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Symposium on
MEDICATED FEEDS

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FISHERIES AND FOOD,
LASSWADE.

SYMPOSIUM ON MEDICATED FEEDS

Symposium on
MEDICATED FEEDS

Proceedings of the
SYMPOSIUM ON MEDICATED FEEDS

Sponsored by
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE,
FOOD AND DRUG ADMINISTRATION, VETERINARY MEDICAL BRANCH
JANUARY 23 and 24, 1956

Chairman
CHARLES G. DURBIN, V.M.D.

Edited by
HENRY WELCH, PH.D., and FELIX MARTI-IBAÑEZ, M.D.

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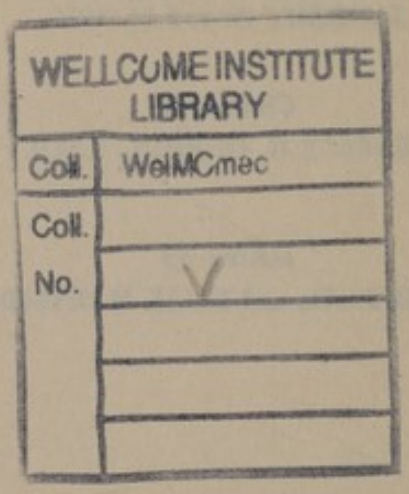

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President's Message

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The White House Washington DC Jan 19 1113ame

Dr. Charles G. Durbin, Chairman Symposium on Medicated Feeds

Food and Drug Admin Room 3220 Hew Bldg

Our nation's agricultural production has received great support from all those who have contributed to the development of improved feeding methods, of lower production costs, and of new and more effective drugs to prevent and treat diseases of livestock and poultry. The extensive and increasing use of medicated feeds supplied by a great and growing industry is an important part of these developments and a boon to small scale as well as large producers of livestock.

In recognition of these accomplishments, which benefit all Americans, I am happy to extend best wishes for the success of the Symposium on Medicated Feeds sponsored by the Food and Drug Administration, Department of Health, Education, and Welfare.

Dwight D. Eisenhower

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Opening Remarks

CHARLES G. DURBIN

Associate Veterinary Medical Director
Food and Drug Administration
Washington, D. C.

It is a real pleasure for me to open this Symposium on Medicated Feeds.

During the last few years, we have seen a tremendous increase in the use of medicated feeds for livestock and poultry. Because of this development, feed manufacturers, with reluctance, have become drug manufacturers. This trend has caused confusion among feed manufacturers, basic drug manufacturers, control officials, and, last but not least, the farmers.

It is hoped that this symposium will aid in clarifying many of the perplexing problems thus created. As you will note, we have a very full program arranged for the two days and it is of the utmost importance that speakers keep within their allotted time. Time has been allowed for a few questions at the end of each paper. We must request that such questions be pertinent to the paper presented.

There will be a panel discussion Tuesday afternoon, moderated by Dr. Henry Welch. At that time, further opportunity for questions will be given. However, we request that you present such questions in writing so that they may be in turn presented to the panel. At this time, I wish to introduce Dr. George P. Larrick, Commissioner of Food and Drugs.

A Word of Welcome

GEORGE P. LARRICK

Commissioner of Food and Drugs
Department of Health, Education, and Welfare
Washington, D. C.

This is a significant and even historic day for the Food and Drug Administration and, I believe, equally so for you representing the drug manufacturers, feed manufacturers, veterinarians, the state feed control officials, and other officials in agriculture. Never before, to my knowledge, has a group such as this had the opportunity to join together and discuss a national problem of mutual interest and concern to all of us. It is important that this be done, and that it can be done, in an atmosphere of professional and business good will.

There has long been too much confusion in the minds of some concerning the role of enforcement officials and the regulated industries. Many still regard our relationship much as a game of cops and robbers. While legal enforcement actions and supporting investigatory procedures will probably always be needed, it nevertheless is becoming increasingly apparent that meetings and joint educational activities, such as this, go a long way toward providing an understanding of the problems, their solution, and a sound basis for voluntary cooperation and compliance. The latter is a major objective of the Food and Drug Administration and the Federal Food, Drug, and Cosmetic Act.

This symposium is but one of many manifestations of the growing importance of medicated feeds. I should like, if I may, to take a minute to emphasize the national importance of medicated feeds. As all of you well know, they present numerous highly complex scientific and technical problems. There are also major economic considerations involved, not only for the manufacturers but for the farmer, the meat packer, and the consumer. Some of these have not yet been fully answered. But over and above all of these, however, looms one paramount consideration. It must govern your actions and it will govern ours. It is the basic concept of all food and drug legislation—the protection of the public.

Medicated feeds cannot be viewed only in terms of their economic or medical effects on poultry, pork, and beef. We, and you, must primarily be concerned with the question of whether or not the treated meat has any discernible adverse effects on the human consumer. Our nation's food supply is fundamental to our nation's health and therefore its strength. These are not matters to be taken lightly. On the contrary, these are matters demanding of our best scientific skills, our best educational efforts, and our best enforcement standards.

As I reviewed the program for the next two days, I was gratified to see speakers representing the manufacturers, the Department of Agriculture, the Food and Drug Administration, the Public Health Service, the state feed officials, the Association of Official Agricultural Chemists, and the feeders. This, in my judgment, bids well for a successful, fruitful meeting. On behalf of the Food and Drug Administration, I extend my compliments and my sincere hope that this symposium will contribute significantly to the health and welfare of our nation.

A Message of Welcome

BRADSHAW MINTENER

Assistant Secretary
Department of Health, Education, and Welfare
Washington, D. C.

Secretary Folsom has asked me to bring to you his warm personal greetings and wishes me to express his regrets that previous commitments made it impossible for him to be with you today. We all appreciate your taking the time from your busy lives to come to Washington to meet with us and to discuss some of the problems confronting both Government and industry in this important area, which is the basis of the program for this symposium.

Having followed the rapid development of medicated feeds over the past five years, both in private industry and in Government, I have a special interest in this symposium. Having been General Counsel for one of the large feed manufacturers, I am fully aware that this is truly a significant event. The Food and Drug Administration has brought together representatives of the livestock and poultry farmers, the feed and pharmaceutical industries, and members of State and Federal agencies to discuss mutual problems arising from the tremendous increase in the use of medicated feeds for animals. These many new regulatory and manufacturing problems can only be met through the close cooperation of all concerned.

It is particularly significant that this symposium should take place during the celebration of the Fiftieth Anniversary of the passage of the first Federal Food and Drugs law. There can be no more fitting demonstration of the success of food and drug legislation, and the progress made in its administration, than this cooperative effort on the part of all concerned not only to share the fruits of scientific advances, but also to meet the challenge of increased public health protection attendant on such advances.

During the 17 months in which I have been a part of the Federal Government, I have been asked many times what are my principal impressions in coming from industry into the Federal Government. Two important impressions have been made upon me during this period. The first is the tremendous size of the Federal Government. The Department of Health, Education, and Welfare includes about 44,000 people, 10,000 of whom are in Washington and 34,000 are in the field. Our annual budget is in excess of 2,600,000,000 dollars. That is indeed big business, both from the standpoint of personnel and from the standpoint of the budget. Our Department, in my opinion, to a greater extent than any other Department in the Federal Government, affects every man, woman, and child in the nation every day of the year. The second impression that has been made upon

me is of the wonderfully fine group of people with whom I am privileged to work in the Federal Government. I have never worked with a more conscientious, competent, experienced, and dedicated group of people in my life. This is a very gratifying and important revelation to me because this kind of a basic civil service is essential, in my judgment, not only for the operation of the Federal Government but also for the preservation of our democratic system, and as long as our Government is in the hands of this type of civil servant, it is in good hands for a long time to come.

I hope that you will find this symposium to have been well worth your while. It is a great pleasure to welcome you here to Washington and to wish you all a most pleasant, interesting, successful, and profitable meeting.

Introduction

CHARLES G. DURBIN AND JOHN H. COLLINS

Veterinary Medical Branch, Division of Medicine, Food and Drug Administration, Department of Health, Education, and Welfare Washington, D. C.

There is remarkably widespread recognition of the fact that the American farmers face profound changes in feeding and medicating livestock and poultry. These changes are the direct result of modern discoveries concerning nutrition, disease control, and the availability of many new drugs for animal use.

The prevalence of animal disease is an important limiting factor in the livestock industry of any country. Failure to develop and maintain adequate disease control measures can lead to heavy annual loss of livestock resources and contribute to high production costs, which in turn may seriously affect the general economy and welfare of a nation. Anyone who keeps livestock on a small or large scale may confidently expect to profit by applying modern feeding methods and disease prevention practices in his daily farm operations. This inevitably leads to the use of drugs in feeds.

It is reported that more than half the feed-lot cattle in this country are now getting diethylstilbestrol in their feeds to increase weight gains, just one year after the introduction of this new use of the drug. Furthermore, enough antibiotics were sold last year to medicate three fourths of all manufactured feeds.

Because of this unprecedented increase in the use of drugs in animal feeds, it was deemed advisable to bring together a group of people interested in medicated feeds to discuss the mutual problems. This thinking was further precipitated by the increased volume of questions received by the Veterinary Medical Branch, Division of Medicine, Food and Drug Administration, regarding the addition of drugs to feeds. When drugs are added to animal feeds for the prevention and treatment of various diseases and/or for promoting growth, such articles become drugs as defined by the Federal Food, Drug, and Cosmetic Act. This has created many new problems for feed manufacturers, drug manufacturers, and regulatory officials. The following general outline was used in formulating the program for the Symposium on Medicated Feeds:

I. History and general remarks.
II. Types of medicated feeds.
III. The pharmacologic aspects of medicated feeds.
IV. Assay and mixing problems involved in the manufacture of medicated feeds.
V. Public health significance.

VI. State regulations.

VII. Relationship of the Food and Drug Administration to medicated feeds.

VIII. Future of medicated feeds.

About 400 people, representing drug manufacturers, feed manufacturers, veterinarians, state feed control officials, and state and Federal agricultural officials, as well as representatives from such countries as Canada, Denmark, France, the Philippines, and others, attended the two day symposium and heard 31 papers and a panel discussion based on this outline.

The spirit of enthusiastic cooperation demonstrated by those in attendance was most gratifying to the Food and Drug Administration.

History of Medicated Feeds

J. E. HUNTER

Allied Mills, Inc., Libertyville, Ill.

Spectacular advances in livestock and poultry feeding have been made during the past few decades. As an example, because of improved nutrition, breeding, and management, chickens and turkeys are now growing almost twice as fast on about half as much feed, as compared to 25 years ago. Medicated feeds have, within the last few years, played an important role in livestock and poultry production, and particularly so where animal or poultry populations are highly congested. The use of medicated feeds as we view them today is quite new, but numerous interesting early attempts were made to prevent or control diseases with medicated feeds. A few of these early attempts at medication through feed will be recounted in this paper.

No complete record can be found of all of the early attempts toward feed medication, and much of the material in this presentation was obtained by personal communication with individuals who for many years have had an opportunity to observe medication of livestock and poultry via the feed route.

About 30 years ago, workers at Michigan State College published reports on colloidal iodine preparations for worm removal and for coccidiosis control with poultry. These iodine products were, in the main, administered directly to birds or added to the drinking water but were sometimes added to feeds on the farm. Colloidal iodine was also used for blackhead control.

Another early use of a medicinal product in feed was a formula for a poultry enteritis powder that originated at the University of Connecticut and that was recommended as a treatment for general weakness, coccidiosis, worms, and miscellaneous conditions. The mixture, the formula of which was published as early as 1928, consisted of powdered catechu, powdered calcium phenolsulfonate, powdered sodium phenolsulfonate, and powdered sulfate of zinc. This formula was generally recommended for use in drinking water but was also used to some extent in the feed. The first commercial usage of this formula in feeds with which this writer is familiar occurred in 1933.

The early thirties saw some commercial usage of finely ground tobacco dust incorporated in feeds for the control of roundworm infestation in poultry. This practice was later replaced by the use of nicotine compounds. Sodium fluoride came into use in feeds about 1934 and is still used as a worming agent for swine.

During the middle 1930's, finely powdered sulfur was mixed into feeds and was rather widely used as a preventive measure against coccidiosis in poultry. The mixture was effective for the purpose for which it was intended but, unless carefully used, rickets was likely to be the end result.

Numerous regulators and panaceas were offered during the 1930's and perhaps earlier as cures for diseases of livestock and poultry. These items were not generally mixed in commercial feeds at the point of manufacture but, if administered to animals via the feed route, were usually mixed at the local level.

Medicated feeds began to receive widespread publicity and public acceptance with the advent of effective coccidiostats for feed use, during the latter part of the 1940's. These products, when properly used, are extremely useful in reducing losses from coccidiosis in poultry. Today's list of medicated feeds is impressive and effective. Included in this list are: antibiotics, at nutritional and therapeutic levels; arsenicals, at nutritional and therapeutic levels; antioxidants; blackhead cures and preventives; hormones; and drugs that aid in controlling *Salmonella* organisms. Cadmium compounds have become widely used as worming agents for swine, and piperazine compounds are beginning to receive acceptance as worming agents for swine and poultry. Phenothiazine has been used for many years in feed as a worming agent for several classes of livestock and poultry and is currently receiving considerable attention as a worming agent for ruminants.

The use of medicated feeds will undoubtedly increase as new drugs are developed and their effectiveness in feeds demonstrated.

Those of us concerned with the use of medicated feeds, whether we are pharmaceutical manufacturers, feed manufacturers, state regulatory officials, or representatives of the Food and Drug Administration, should be most conscious of our responsibilities in this connection and should carefully assure ourselves that any new medicinal items to be used in feeds will produce the desired beneficial effects without accompanying adverse effects that might overshadow the good derived from the use of such agents. In our instructions to the feeding public, we should always stress the fact that, regardless of the effectiveness of medicated feeds, they can never substitute for good management of livestock and poultry.

Medicated Feeds, Some General Comments

R. E. LUBBEHUSEN

Ralston Purina Co., St. Louis, Mo.

One might point with justifiable pride to the rather amazing advancement of the past decade as measured in terms of efficiency of production in both livestock and poultry. While breed improvement and research in nutrition have made major contributions, let us not be unmindful of the fact that concurrent strides in more effective disease control helped to make these records possible. Indeed, the optimum in nutrition results can only be attained with animals whose functional capacity is unimpaired by disease. Therefore, the health status of the individual and hence of the herd or flock has always been a matter of vital interest to the nutritionist and the mixed feed industry.

The inclusion of additive materials to the ration for the express purpose of preventing or even treating specific types of pathologic changes has marked many of the advances in nutrition research. Although they actually function as medicaments, these additives, such as certain of the vitamins, minerals, and trace elements, have long been classified as dietary essentials.

Depending upon one's personal viewpoint, the biggest success story or the biggest headache in the history of the feed industry has been the extension of this sound principle of additives from the nonspecific disease realm over into the field of infectious diseases. How did this transition come about, and why do we find ourselves in a situation today that makes this symposium, with its free exchange of viewpoints, so timely and important? I think the answer is quite simple. After years of rather indifferent success via the feed approach, research in the field of disease control finally resulted in the discovery of a chemical that was highly effective in reducing the clinical incidence of a widespread and devastating disease in poultry. The chemical that gave the tremendous impetus to feed medication was sulfaquinoxaline and the disease was and is coccidiosis. Other chemicals for the same and other disease conditions have followed in rapid succession. Collectively, their number and proved record of efficiency represent a truly amazing research accomplishment. But let us get back to our simple answer as to why the feed mixer has had to assume the role of pharmacist and then incidentally ventured into what was presumed to be the field of veterinary medicine. Almost all of these medicinals shared two things in common, namely, their dosage even at treatment levels was exceedingly small and, secondly, few were water soluble. The former called for thorough blending in a mass medium and the latter, i.e., relative insolubility, pointed to feed as the carrier of choice. Add to this the further fact that mass medication has always had a strong appeal to the feeder because

of its labor-saving aspects, and you have the background of a strong motivating force. And what has been the result? If you are in the mixed feed business today, you have been forced to assume the responsibility of thoroughly blending exceedingly small amounts of medicinals in certain of your feed bases; you are clearing, labeling, registering, and selling these products not as feeds but as drugs; and, furthermore, you are assuming the responsibility for results in a field that requires a basic knowledge of the fundamentals of disease control.

The true service value of any medicated feed service program must be based on ability to deliver the right medicinal agent at the right level at the right time and for the right purpose. This is rather an exacting assignment, and yet we have come through these past few years with such a remarkable record of accomplishment in controlling certain infections via the medicated feed route as to convert even the most skeptical to the basic soundness of this approach. It may be well to remember, however, that this record was made in the field of preventive rather than therapeutic medication. It was made because research resulted in the finding of chemicals of outstanding efficiency in preventing clinical outbreaks of several infections that represented universal problems of the poultry industry; hence, the probable need for preventive medication could be projected with a fair degree of accuracy in the light of past experience. The record was made because such chemicals had a fair margin of safety and could therefore be included in the feed mix with a minimum hazard of toxicity. Lastly, but by no means least, the results attending the preventive program usually outweighed the increased cost of such medication. The situation that favored the introduction of the preventive medicated feed approach and that has contributed to its continuing success might well be described as a "natural."

It would be as naive as it is untrue to imply that preventive medication via either the feed or water route has been completely successful and that we have not and do not now face serious problems. Experience of the past several years would certainly indicate otherwise. The optimum in effective disease control through preventive medication is only attained when each contributing force meets its particular responsibility. These forces are: research, which discovers the drug; engineering techniques, which translate these discoveries into the commercial product (medicated feeds); the individual who recommends it; and the customer who uses it.

As indicated previously, research can point with justifiable pride to the screening studies that revealed the basic utility of certain chemicals in the disease control field. However, there have been those occasional instances when pride or exuberance appear to have been responsible for the premature release of publicity concerning favorable screening test results. This has often stimulated a consumer demand in advance of other vital information or proved performance under field conditions. The customer request for an unproved medicinal agent at a still debatable level on a special personal mix basis has been an all too common problem. Fortunately, this situation is showing some evidence of improvement. While there may still be some tendency to premature action because of competitive pressures, most of the basic producers of the medicinal additives now maintain comprehensive research setups for evaluating product performance under practical field conditions. Not only that, but many have also embarked upon extensive programs of cooperative research with Agricultural Experiment stations and those of the

mixed feed industry who are in a position to assist in such critical evaluations. Such critical studies not only apply to the matter of drug efficiency but also to the safety and adaptability to the feeding program. It should be remembered that whether a feed formula contains a medicament or not has nothing whatsoever to do with its primary objective, namely, that of supplying the nutrients considered as essential to the functional requirements for the period under consideration. Not in any sense is the feed base ever to be regarded as being inert, and it further behooves us to be certain that the medicament does not interfere with the nutritive program. In other words, it is imperative that we weigh the advantages of medication against its adverse effect, if any, as measured in terms of a lowered feed intake, reduced feeding efficiency, or a lowering of production. Investigations of this nature are as time consuming as they are necessary in providing a full measure of protection to the customer. After the basic and applied research is completed, one is then faced with the task of translating technical information into program uses that can be understood and put into practical application by the customer.

If misuse of products and a misconception of what each will accomplish are criteria, we must admit that our educational efforts leave much to be desired. Witness the field complaints on suspect coccidiosis breakthroughs, and you will find ample evidence that, after some years of coccidiostat experience, many poultrymen, and even those in field service work, still do not understand the protective principle involved or the limitations of such products. It must be apparent that label or tag directions for use are not enough and that a continuing educational program must be carried on at the customer service level. It is my humble opinion that the future of medicated feeds as a service approach to disease control will depend upon the extent and soundness of the educational programs that are developed. The customer must clearly understand what may reasonably be anticipated in the way of results if the product is used as directed.

And what of the responsibility for medicated feed results? It is elementary to say that the "inert" agent carries the responsibility for nutrition results and the "active" one that of animal health. Except under other than carefully controlled split test conditions, it becomes exceedingly difficult, if not impossible, to evaluate the exact extent to which either may have contributed to the well-being of the animal on the one hand or lowered feeding efficiency on the other. The latter is particularly true when the morbidity symptoms are nonspecific. I would not pretend to have the answer as to the relative extent to which the drug supplier and the feed mixer should share the liability for the poor results that may attend the correct use of a medicated feed, but possibly we may have a basic situation somewhat analogous to that of the physician-pharmacist relationship in human medicine. Provided with a prescription designating a certain drug at a definite dosage, it becomes the pharmacist's responsibility that the medicament meets such specifications. This responsibility ends there. Provided with information to the effect that a certain drug at a certain dosage has been approved for inclusion in a feed base, it is the feed mixer's responsibility to supply the medicament thoroughly blended in the inert agent of choice at the tag designated level. That responsibility is clear-cut and unmistakable. Less clear, however, are the reasons why the feed mixer's liability should extend beyond this point and include product performance as applied to the prevention or control of clinical outbreaks of disease. Unless he carries through on an extensive research program of his own, all he

actually provides are the mechanical facilities of blending the drug on someone else's recommendation for purposes that are foreign to his field of training. Competitive pressures may leave him without much of an alternative, but, nevertheless, his medicated feed mix is frequently held accountable for results unrelated to nutrition.

Conversely, the medicament should not be expected to compensate for an inferior ration in the over-all results. The ration should not only meet the dietary needs, but it should likewise be free of materials that might be incompatible with the drug. This matter of compatibility requires the cooperative research approach. Without belaboring the point any further, the need for a clearer definition of the responsibilities of the drug supplier and the medicated feed mixer in their mutual contacts with the feeder should warrant some further discussion. Important as the relative responsibilities of the drug suppliers and producers of medicated feeds may be, the constructive efforts of either group are often impaired or completely nullified by the feeder. I am referring to (1) his use of the wrong product because of a misconception as to the identity of the disease problem; (2) his use of the right product at the wrong time; (3) his failure to follow directions as they apply to dosage, such as making medicated and nonmedicated feeds available to animals concurrently on a free choice basis; (4) his failure to heed toxicity warnings as applied to species or age periods; and (5) his failure to recognize the practical limitations of any medicament in an over-all disease control program.

Whatever the cause of failure, the customer often appears to have developed the erroneous impression that preventive medication is the sole answer to his disease control problems. Those management and sanitation practices that once were such an important part of his control program are now considered by him to be nonessential. As a matter of fact, there are even those who encourage a disregard of sanitation in order to assure exposure adequate to the buildup of immunity. For the protection of the customer as well as the supplier, every effort should be made to keep the disease control limitations of medicated feeds in their proper perspective. The customer must be made to realize that preventive medication via the feed or any other route merely offers an additional means of protection and that adequate disease control programs must still include proper management, strict attention to sanitation, proved vaccination procedures, and a well-balanced nutrition program.

It may be well to temper our enthusiasm concerning our service accomplishments in preventive medication in the light of the increasing and alarming trend toward the use of medicated feeds in the treatment of disease outbreaks. This trend could be a dangerous one marked by disservice to the customer and reflecting discredit to the mixed feed industry whose success has always been so intimately tied to the customer. Preventing a conflagration is one thing, but stopping it is quite another. We may have a strong desire to put out fires, but let us be mindful of our fire fighting ability. No one can deny our technical knowledge of blending treatment, as well as preventive, levels of a drug into a selected feed base. The problem is to have the proper blend at the right place at the right time with full realization of what this implies. Unlike preventive medication, the specific need can seldom be anticipated, and delay is often tantamount to disaster. Before we decide to include drugs at treatment levels in our feeds, let us ponder the fact that the effectiveness of any drug for treatment, regardless of its method of

administration, is predicated upon a prompt and critical observation of symptoms, an accurate diagnosis, and immediate treatment thereafter if mortality and morbidity losses are to be kept to a minimum. In other words, it is imperative that we identify the problem! Obviously, this calls for the ready availability of those with veterinary training or its equivalent at the local level. Any attempt to circumvent this basic requirement immediately places us in a vulnerable position of liability, and it also poses a problem of public relations with a profession that is essential to the maintenance of animal health and, hence, to the success of our business.

When the untrained individual prescribes treatment for an undiagnosed disease outbreak on the theory that "it won't do any harm even if it doesn't do any good," and if he does so without advising that he is following the trial and error method, he is treading on the dangerous ground of possible disservice to the customer. He might guess correctly, but mistakes are costly as measured in terms of unnecessary mortality losses and the added outlay for ineffective medicaments. Perhaps most critical of all is the delay in implementing sound control procedures for the multitude of those diseases not amenable to treatment via the medicated feed route. A field report that has just come to my attention is a case in point. The locale was southern Ohio, the problem a disease outbreak in a group of approximately 2000 turkeys 22 weeks old. Symptoms of depression and a decline in feed consumption were noted with the first mortality loss the following day. The service man suspected cholera and recommended appropriate treatment, but the mortality rate increased. Two days later bluecomb became the suspect, and a high-level antibiotic feed was next on the list. After the passage of five more days and the death of 415 birds (valued conservatively at $2000), representative specimens were submitted for laboratory examination. The diagnosis was erysipelas, the treatment given was an injectable antibiotic, and the mortality stopped within 36 hours. At what point in this case did the service contact begin? It began when the problem was positively identified as the basis for constructive action. Prior to that time, every move, however well intentioned, was one of disservice to the customer.

As anyone with extensive field experience must admit, instances comparable to this are rather commonplace. We should never be unduly critical of the honest effort to be of service but, when in doubt, honesty should require an understanding, crystal clear to the customer, that recommendations for such medication are in the nature of a first aid attempt until a definite diagnosis can be established and that the latter steps be implemented immediately and not as a last resort. Although it is a veterinarian's responsibility, it is not necessarily our concern who makes the diagnosis just as long as it is based on a correct evaluation of facts. The concern of a veterinarian over the misdirected diagnosis and treatment by those who are not qualified is understandable. It stems from a sense of his responsibility for the livestock health of his community and it must also, in part, stem from a sense of frustration. I have no intention of pleading the veterinarian's cause. To do so would be as presumptive as it is unnecessary. The profession is well able to speak for itself and can, on occasion, do so with considerable force. While mutual contacts with our customer, who is his client, may occasionally precipitate some friction, I for one refuse to regard it as a major problem. Much misunderstanding would be avoided by a simple recognition of the veterinarian's right to evaluate the feeding program as an important factor in animal health and of our basic

interest in disease as it may adversely affect feeding results. Both groups have a public relations responsibility that has been sadly neglected for the most part. A well-informed veterinary profession is our staunchest ally. It has a right to be, and it is, interested in our products (particularly medicated feeds) and their programs of use.

We have not done a particularly good job of keeping the veterinary profession fully informed concerning current developments in nutrition and the medicaments applicable to feed administration. Bear in mind that the veterinarian is certainly aware of the growing popularity and the basic soundness of mass administration for certain types of medication. He must and no doubt will adapt himself to the profound changes that are taking place in the field of preventive veterinary medicine. These changes are rapidly obliterating some of the old boundaries of relative responsibility in the disease control field. The basic question as to who shall do what is not one of prior right but of ability to serve. The manufacturers of the additive drug, the producer of the medicated feed mix, and the veterinarian share the common responsibility of maintaining the optimum in animal health and productive efficiency at a minimum of cost to the American farmer. The national organizations of each group should intensify their cooperative efforts to that end.

Laboratory Rations and the Feed Manufacturer's Responsibilities

JACK M. CURTIS

Division of Pharmacology, Food and Drug Administration
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Laboratory rations used for the maintenance of small animal colonies in research laboratories constitute only an insignificant portion of the amount of animal feed that is prepared throughout the country. The importance of these laboratory rations is far greater than is indicated by their dollar value. A great number of research projects are dependent on the reproductive capacity of laboratory colonies, which in turn is dependent upon an adequate, uncontaminated laboratory ration. Furthermore, a great many clinical tests, such as estrogenic bioassays and pregnancy tests, and hormone and vitamin research are dependent upon an abundant supply of laboratory animals that have been maintained on a diet in which the experimenter has confidence. These laboratory rations do not come under the heading of medicated feeds, but they are discussed here because they sometimes become contaminated accidentally by the medication that is put into other feeds. Consequently, any operation that interferes with the regular supply or well-being of laboratory animals becomes of considerable importance and it is our interest to prevent this interference.

On several occasions, it has been brought to the attention of the Food and Drug Administration that laboratory rations have been contaminated accidentally by stilbestrol. This contamination is not only of the finished, mixed feed but also of ingredients that have been sold as factors to be mixed in a feed by the experimenter himself.

To my personal knowledge, one investigator is having to repeat almost a year's research on a vitamin project because, toward the end of the experiment, estrogenic stimulation from the diet became evident. In another case, one of the ingredients purchased to make a mouse diet apparently had been processed in a mill immediately following the preparation of a stilbestrol-containing steer feed. This ingredient was so heavily contaminated with the drug that even after dilution with other ingredients, there was sufficient estrogen to destroy the breeding capacity of a mouse colony.

Obviously, such a contaminated feed is adulterated under the terms of the Food, Drug, and Cosmetic Act. It is equally obvious that the New Drug section of the Act is not applicable.

The Food and Drug Administration does not contemplate, at this time, an extensive program of examination of laboratory rations to determine the extent

of this contamination. Contamination, when it occurs, comes to our attention after the damage has been done to a colony of animals. Consequently, if we can prevent this contamination from occurring by informing all those making laboratory rations of the hazards and insisting upon adequate control procedures, we have accomplished much more than by initiating legal action after injury has occurred.

It seems probable to me that most of the feed mixers or millers are unaware of the injury that may be produced by very small quantities of stilbestrol. Two parts/billion of stilbestrol in a mouse diet will cause measurable growth in the uterus of an immature mouse and 10 parts/billion will produce continuous estrus. The addition of only 0.9 pound of a steer feed supplement containing 10 mg./lb. of the drug, when mixed with 1 ton of mouse food, produces a concentration of 10 parts/billion in the mouse diet. Therefore, the industry should be informed of the unusual precautions necessary in cleaning equipment used to prepare stilbestrol supplements before other products can be processed. This is the only way to preserve the integrity of special diets.

The information that is available to the Food and Drug Administration relates to the safety of estrogen-containing diets only in steers and chickens. We also have information concerning the injurious capacities of these diets to laboratory rodents. We do not know whether or not it is safe to feed other domestic animals, i.e., swine or sheep, this drug. We believe that research on these latter animals is now in progress.

I should like to propose that an educational program be developed cooperatively by the Food and Drug Administration and holders of primary new drug applications of stilbestrol supplements to inform various feed mixers and millers of the extent of the damage that might be caused by contamination of laboratory feeds with stilbestrol and to make recommendations as to the extreme care that must be taken to prevent contamination.

The value of such a program, when measured in terms of reducing the loss of experimental results and in terms of promoting public good will, will be many times that of the value of the laboratory rations produced.

Hormones in Feeds

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Interest in hormones in animal feeding has flared since November 1954, when the Food and Drug Administrator permitted an application to become effective for the feed use of 10 mg. daily of diethylstilbestrol for fattening cattle weighing 600 pounds or more. Estimates of the number of cattle fed this drug since that date are of the order of 5 million. Additional applications for the feed use of diethylstilbestrol for fattening cattle have been permitted to become effective, as have applications for feed use of dienestrol diacetate for meat-type poultry. Also of continuing laboratory interest are 1-thyroxine, iodinized protein, and thiouracil, although feed use is limited.

An excellent review of the earlier research was published by the National Academy of Sciences, National Research Council, in 1953.[1]

The early interest in hormone feeding of poultry was intense. Lorenz[1] summarized this research as indicating that the stilbenes, diethylstilbestrol, hexestrol, and dienestrol, and their dimethyl ethers, respectively, dianisylhexene, dianisylhexane, and dianisylhexadiene, show the following decreasing order of potency when fed to chicks: dianisylhexane, dianisylhexene, dienestrol, hexestrol, dianisylhexadiene, and diethylstilbestrol. The oral potency of dienestrol diacetate for the chicken is identical with that of dienestrol. Oral administration of diethylstilbestrol to poultry generally gave unsatisfactory results. This drug, administered as a single 12 mg. pellet implanted under the skin at the back of the head, in accordance with an application permitted by Food and Drug Administration to become effective in 1947, has been used to improve the finish of many millions of meat chickens.

While there is no tissue retention of diethylstilbestrol or dienestrol, used in the permitted manner, the dimethyl ethers show highly undesirable tissue retention characteristics and their use has not been permitted.

Estrogen-treated poultry, turkeys to a lesser degree than chickens, show more subcutaneous fat and therefore better finish than their controls. Breast muscle fat content may be doubled with consequent improvement in juiciness of the meat. Color of flesh is lightened and tenderness increased. Increase in growth rate of estrogen-treated poultry is small and of little economic consequence.

Physiologically, the principal effects of estrogens on poultry are the anticipated effects of feminization. Effects are generally proportional to oviducal response. The effects include blood calcemia, lipemia, and proteinemia. Some hyperossification takes place. The size of the comb and of the testes is greatly depressed.

Masculine behavior disappears. Exceptionally, however, especially in young turkeys of both sexes, treated birds exhibit exaggerated male behavior during the first few days after treatment is initiated. The relationship of estrogens to egg production is incompletely understood. It is probably one of a chain of hormones involved in the normal ovulation cycle; a single dose will interrupt broodiness. High, continuous dosage interrupts egg production. Feathering, in the sense of lack of pin feathers, is improved. There have long been indications of estrogen-thyroid interrelationships but these are still ill-defined.

F. W. Hill[1] summarized the earlier work on thyroid application in poultry feeding. Andrews and his co-workers[1] found that a 12 mg. stilbestrol pellet largely reversed the depressing effect of 0.15 to 0.2 per cent of thiouracil in the feed on growth and feed consumption, permitting maximum broiler fattening. Winchester[1] summarized the research on thyroid-active compounds and reported that he and his associates found that thiouracil-fed pigs kept at 50 F. environmental temperature produced hams with 14 per cent less fat than their controls and 6 per cent more protein. At higher environmental temperatures, the differences found were not significant. The use of stress factors in studying the physiology of hormone action will be used more in the future.

You are all familiar with the work of Turner and his colleagues at the University of Missouri, which showed that milk and egg production might be stimulated by iodine feeding. There is some division of opinion with respect to the practical value of feeding thyroactive protein. Swanson[2] reported that a 15 day withdrawal period was adequate to prevent a terminal drop in milk production below normal levels. However, Thomas and Moore[3] have demonstrated to our satisfaction that net milk production over the entire lactation period is not profitably increased by feeding thyroprotein.

However, it is quite certain that the activity of the thyroid is a critical factor in the growth and fattening of animals, in lactation, and in egg production. There is and probably will continue to be a lively research interest in the effects of modifying thyroid activity on growth and production.

Shaklee and Knox, of the Animal and Poultry Husbandry Research Branch at Beltsville, Md., have demonstrated a high heritability of thyroid weight in young New Hampshire chickens. The correlation between thyroid weight and body weight at 4 weeks of age was $r = +0.59$. Now thyroid weight and thyroid activity may be negatively rather than positively correlated. More research must be done. In dairy cows, too, a cooperative project among a considerable number of State Experiment Stations and the Dairy Husbandry Research Branch[4] disclosed that, in general, cows in the North have heavier thyroids than cows in the South. Again, we are not prepared to appraise the significance of the finding.

Kunkel et al[5] suggested that the blood protein-bound iodine level may be indicative of capacity for feed-lot gain in beef cattle. Gawienoski et al[6] reported data that show a marked relationship between average daily gain of Hampshire pigs to 215 pounds live weight and protein-bound iodine in the blood serum at time of slaughter. Expressed as micrograms of iodine per 100 ml. of serum, protein-bound iodine values of less than 2 mg. were associated with average daily gains of about 1⅓ pounds; protein-bound iodine levels of 2 to 4 mg. with average daily gains of about $1\frac{1}{10}$ pounds; and protein-bound iodine levels of 4 to 5.5 mg. with average daily gains of about $\frac{9}{10}$ pound.

A review on the clinical significance of blood iodine by Rapport and Curtis[7] suggested that protein-bound iodine in the serum may be a better index of thyroid activity than basal metabolism. There will be more research on thyroid activity and inherent growth capacity, but determination of protein-bound iodine is not simple.

Several papers have reported research on the interrelation of thyroid activity and the growth-promoting effects of low levels of antibiotics. Barber et al[8] reported experiments in which 46 pound weanling pigs were put on control, antibiotic supplemented, and antibiotic and 1-thyroxine supplemented diets for 112 days. The average daily gain for the controls was about 1.2 pounds; for the pigs given antibiotic supplement, about 1.3; and for those fed both antibiotic and 1-thyroxine, 1.4 pounds. The corresponding pounds of feed per pound of gain were about 3.65, 3.55, and 3.45, respectively.

Mellen and Waller[9] reported that feeding chlortetracycline or bacitracin increased the thyroid weight of chickens. Menge and Conner[10] also reported that the addition of chlortetracycline in the diet of chicks increased the size of the thyroid gland at 4 weeks of age. Now these reports by no means provide a demonstration that the growth-promoting effects of antibiotics are mediated through the thyroid, but the role of the thyroid will certainly receive active research attention and may prove to be substantial.

The earlier studies of the effects of estrogens and androgens on swine and ruminants laid the groundwork for the present surge of research on feeding them. A great deal of work has been done with pellets, especially of diethylstilbestrol. The standard 12 mg. pellet was available, effective, and conveniently applied. The results in cattle and sheep were dramatic but quite unlike those with poultry. Large growth responses resulted from stilbestrol implantations but no acceleration of fattening. Some positive results were obtained with swine with respect to growth but not generally. Teat growth in swine was found regularly and sufficiently to cause concern. One interesting report by Braude,[11] to which we will return, contained indications that ratio of growth and feed efficiency might both be increased by combined treatment with stilbestrol and iodinated protein.

Research with testosterone by Dinusson et al[12] and by Bogart et al[13] resulted in increased rate of gain in steers and heifers. Other workers reported little or no effect.

The consistent research finding of lowered carcass grade and obvious stimulation of accessory sex organs has dampened interest in the use of diethylstilbestrol pellets for cattle and sheep. It was not until Burroughs et al[14] reported their outstanding results with feeding small amounts of diethylstilbestrol to cattle that interest really blazed.

You are all generally familiar with that work and the many confirmations that have followed. In November 1954, the Food and Drug Administration permitted an application for feed use to become effective and diethylstilbestrol became at once a major feed constituent.

Burroughs et al[15] presented a summary of stilbestrol feeding experiments with cattle, conducted at nine agricultural experiment stations, to the Iowa Cattle Feeders in August, 1955. These reports, from Colorado, Kansas, Michigan, Nebraska, Ohio, Purdue University, Tennessee, Texas, and Iowa, included 255 control cattle and 293 stilbestrol-fed cattle. The average daily gain of the controls

TABLE I

Data Obtained from Farm Reports

Location[18, 19]	Control		Stilbestrol-fed	
	Gain	Feed cost	Gain	Feed cost
4 Illinois farms	2.25	—	2.85	2-6¢<control
1 Kansas farm	—	—	2.4	—
1 Colorado farm	—	—	2.5	—
1 Missouri farm	2.03	—	2.27	9.4%<control

was 1.97 pounds; of the stilbestrol-fed, 2.34 pounds. The average dressing percentage of both control and experimental lots was 60.7. There was no significant difference in carcass grade, both controls and experimental lots ranging from good to choice with an average of high good.

The summary of these experiments indicated an increase in rate of gain of more than ⅓ pound per head per day, a feed saving of 12 per cent, no difference in carcass grade, and no difference in dressing percentage.

A report by Klosterman and co-workers[16] is of considerable interest, although stilbestrol implants were used rather than stilbestrol feeding. Control steers had an average daily gain of 2.08 and stilbestrol-implanted, 2.79. Control bulls gained 2.43 pounds per head per day while stilbestrol-implanted bulls gained 2.74 pounds per day. The increase in gain attributable to stilbestrol was about twice as much in the steers as in the bulls but treated bulls and steers gained at about the same rate. The stilbestrol-treated steers had slightly lower carcass grades than the controls while stilbestrol treatment significantly improved the grade of the bull carcasses.

Mitchell and co-workers[17] recently reported experiments in which control and stilbestrol-fed cattle were carried to constant weight and other stilbestrol-fed cattle were fed to the same total feed intake as the controls. The controls had an average daily gain of about 2 pounds and the experimental cattle fed to the same weight, over 2.5 pounds, so the controls were fed for 124 days and this lot of experimental animals for only 96 days. The control steers required 846 pounds of concentrate per 100 pounds gain while the stilbestrol-fed steers required only 665 pounds. Dressing percentages for the two lots were identical and high, 64.4 per cent. The third lot of steers, fed stilbestrol and held until they had consumed the same total amount of concentrate as the controls, were fed for 125 days and their average daily gain was 2.34 pounds. They weighed 35 pounds per head more than the controls at the end of the feeding period, required 719 pounds of concentrate per 100 pounds of gain, and dressed 63.4 per cent.

The summary of these experiments indicated an increase in rate of gain of more than ⅓ pound/head/day, a feed saving of 12 per cent, no difference in carcass grade and no difference in dressing percentage.

The farm reports, by their nature, are usually uncontrolled and inexact. Generally, they report increased rates of gain. A few from which data have been reported show the results given in table I.

Additional experiment station data not included in the Iowa summary include: work at the Tennessee Station, reported by Bell et al[20] for yearling steers fed cottonseed meal as a protein supplement compared with similar lots of steers fed a supplement in which the cottonseed meal was replaced with nitrogen-equivalent urea with and without 10 mg. stilbestrol/head/day, respectively. The control steers averaged 2.05; the urea steers with stilbestrol, 2.11; and the urea steers without stilbestrol, 1.76 pounds/head/day.

Some additional data of interest with respect to the Texas steers apparently included in the Iowa summary were reported by Slagle.[21] Stilbestrol-fed steers had a shipping shrink of 8.6 per cent compared to 6.7 per cent for the controls. The hide weight of 10 mg./day stilbestrol-fed steers averaged 80.6 compared to 81.5 for the controls, but it is interesting to note that a lot fed 20 mg./day had average hide weights of 89.2 pounds/head.

There are a few reports on antibiotic-hormone combinations and research of this type will surely increase as feeding practice is already doing. Rohlf,[22] reporting at a Pfizer field day, stated that Pfizer tests showed that steers fed both oxytetracycline and stilbestrol gained 13 per cent faster on 6 per cent less feed than those fed only stilbestrol. Feeding trials with lambs fed 3 mg./head/day of stilbestrol and feed containing 10 Gm./ton of oxytetracycline gained 20 to 30 per cent faster than the controls and the carcasses were said to be nearly the same as those of the controls.

A report by Hentges et al[23] on steer feeding trials with stilbestrol, chlortetracycline, and a combination of the two showed no apparent advantage of the antibiotic-stilbestrol combination over stilbestrol alone.

Feeding trial data for stilbestrol-fed lambs, without antibiotics, were reported by Acker et al[24] at the 29th Oklahoma Livestock Feeders' Day. The usual increase in rate of gain as obtained (table II). But 26 per cent of the lambs were classed as yearlings and the pelts were a little heavier and more difficult to remove. This is no specific effect of stilbestrol, for lambs implanted in the permitted manner with the estrogen estradiol and progesterone showed a much higher percentage of lambs classed as yearlings and much harder pelt removal. Estrogens apparently hasten closure of the break-joint and thicken the skins of lambs.

TABLE II

Feeding Trial Data for Lambs

Lot and treatment	Control	0.5 mg. stilbestrol/lb. feed	10 mg. estradiol, 250 mg. progesterone
No. of lambs	35	34	34
Av. daily gain	0.34	0.39	0.49
Feed/100 lb. gain	983	825	696
Carcass yield	51	50	49
Carcass grade	Top good (−)	Top good (−)	Av. good (+)
% classed yearling	0	26	59
% pelt of live wt.	14.2	14.4	15.0
Value per cwt.	19.81	18.17	15.37

Hale and co-workers[25] reported data for individuals and groups of lambs fed 150 to 1200 mg. of stilbestrol per pound of feed. A 600 mg./pound/feed level provides about 1.8 mg./head/day. This level gave a 22 per cent increase in gain in weight and a slight decrease in carcass quality. The highest level sharply decreased carcass quality, gave some bulbourethral enlargement, and caused urine dribbling. In one lot, which received 2.7 mg./head/day plus 3 per cent of fat, the rate of gain, carcass quality, and dressing percentage were all improved and there were no apparent adverse effects.

Jordan et al[26] reported results with lots of lambs fed with concentrate-roughage ratios of 45:55 and 35:65, plus 0, 21.6 mg. chlortetracycline/head/day, 2 mg. stilbestrol/head/day, and both stilbestrol and chlortetracycline, respectively. They reported that: "At neither concentrate-roughage ratio did the addition of antibiotic, stilbestrol, or a combination of the two significantly affect the rate of gain, feed consumption, or feed efficiency." The Lilly Agricultural Research Farm[27] reported that 9 of 10 control gilts bred, farrowed an average of 8.4 live pigs and 1.1 dead pigs compared to 8.4 live and 0.4 dead pigs for 8 gilts farrowing of 9 bred, following stilbestrol-fed cattle. Of interest, too, is the fact that the Dairy Husbandry Research Branch at Beltsville fed 10 mg. of stilbestrol/head/day to pregnant milking cows through about the last half of the lactation period with no apparent effects.

With respect to pigs following stilbestrol-fed cattle, reports are still scanty but provide no present cause for alarm. We know that the feces of stilbestrol-fed steers do have estrogenic activity and we know from research at Beltsville that more than 3 mg. per day of estrogen per open gilt will induce pseudopregnancy and temporary sterility. Apparently, amounts of estrogen ingested by gilts following stilbestrol-fed steers do not reach this level.

Culbertson et al[28] reported that they placed 12 open gilts with stilbestrol-fed steers 20 days before breeding; 6 similar gilts were used as controls. The treated gilts remained with steers and did eat their droppings until the first of them was ready to farrow. They were also hand-fed 5 pounds of feed daily. Eleven of the treated gilts farrowed 103 live pigs, of which 82 per cent were alive at 1 week of age. Five of the 6 control gilts farrowed 29 live pigs, of which 86 per cent were alive at 1 week of age. Obviously, the average of 9.36 live pigs, farrowed by the 11 treated gilts, appears to be completely normal. The twelfth gilt may have been pseudopregnant, for she came in heat eight days after removal from the steer feedlot. The control gilt that failed to farrow showed early pregnancy on slaughter. Hanson[18] reported that an unnamed Kansas feed manufacturer found that bred gilts following stilbestrol-fed heifers averaged 1 more pig than the controls.

Let us return to consideration of the carcasses of stilbestrol-fed cattle. First, is the flesh free of stilbestrol residues? Obviously, the Iowa workers filed with the Food and Drug Administration, in support of the application for feed use, adequate data showing no residues in the flesh of the cattle they fed in the now permitted manner. There is published supporting evidence by Perry et al[29] showing that steers fed 10 mg. daily of stilbestrol show no detectable residue in the flesh as assayed by the mouse uterus test. Perry et al[29] also assayed flesh of steers fed 10 mg./day of the other two stilbenes of interest, dienestrol and hexestrol, and found no residue (table III). The authors noted some increase in teat length and elevation of tailhead in the treated steers, least in the dienestrol-treated.

TABLE III

Mouse Uterus Weights of Female Test Mice Used to Assay Steer Flesh

	Control steers	Stilbestrol-fed steers	Dienestrol-fed steers	Hexestrol-fed steers
Mouse uterus flesh, mg.	19.7	16.3	17.4	15.8

Of great interest are three independent projects conducted in the spring of 1955 by a private breeder in Wisconsin, another at the University of Wisconsin, and one at the U. S. Fur Animal Experiment Station at Cornell University. Each of these experiments was addressed to the question of the possible effects on mink reproduction of very small amounts of stilbestrol, such as might conceivably occur in unwashed tripe from stilbestrol-fed cattle. Previous work, conducted cooperatively by Shackelford[30] at the University of Wisconsin, had shown that daily dosages of 10 μg. per female mink, or more, fed during breeding and gestation periods, would sharply reduce the number of live litters born. The private Wisconsin work, the USDA-Cornell work, and the University of Wisconsin work each included stilbestrol series at dosages below the 10 μg. level. In each case, a dosage of 5 μg. or less produced more, not less, kits per lot than the control. The USDA-Cornell work confirmed the destructiveness of the 10 μg. dosage. We may not conclude that very low dosages of stilbestrol improve mink reproduction but the results do warrant further research on this question. The University of Wisconsin work, cooperatively with the Iowa Experiment Station, also examined directly the possible effects of unwashed tripe from steers fed stilbestrol in the permitted manner. They found no evidence of effect during the 1955 breeding season. Research is continuing at the University of Wisconsin and USDA-Cornell on the possible effects of very low stilbestrol dosages on growing mink.

An ever-present hazard in the manufacture of stilbestrol-containing feeds is that of contamination of other feeds. This hazard became a reality in two instances reported to me verbally from highly reliable sources. In one instance, a mouse assay colony was rendered useless due to stilbestrol stimulation from feed mixed in machinery previously used to mix stilbestrol-containing cattle feed. In the second instance, a rat colony showing estrogen stimulation was fed feed mixed in machinery with the hopper in the same large room as the hopper of machinery used to mix stilbestrol-containing cattle feed. Both these cases were promptly corrected and the feed manufacturing industry was informally advised of the hazard of stilbestrol contamination even in very minute amounts in the feed for exquisitely sensitive laboratory animals.

With respect to carcass quality, reports last spring were very numerous that stilbestrol-fed cattle were not dressing out carcasses of quality equal to their appearance at time of slaughter. Reports of soft eye muscles, watery flesh, and poor marbling were frequent. It seemed necessary for the Agricultural Research Service to collect careful research information on these points under its own immediate supervision. This research is not yet complete but preliminary results can be reported (table IV). One lot of 10 beef steers, weighing about 800 pounds

each and of good slaughter grade, was randomized into two lots of 5 each. One lot of 5 was fed 10 mg. stilbestrol daily in the permitted manner; the other lot served as controls for an 84 day feeding period. A second lot of 600 pound beef steers was similarly randomized and slaughtered about December 1, after a six month feeding period. In addition, one Milking Shorthorn steer was fed 30 mg. and another was fed 60 mg. daily for 151 days.

There were no very obvious external effects on the appearance of the stilbestrol-fed steers, although the teats probably lengthened a little. The two Shorthorns on the high dosages were hard to keep on feed during hot weather. During the final 28 day period, when the weather was cooler, the 30 mg. steer gained 3.7 pounds per day.

The carcasses certainly showed no evidence of adverse effects of feeding stilbestrol in the permitted manner. The color of the flesh of both control and treated lots was cherry red, rather than the preferred light cherry, but both lots were the same. The flesh of both lots was firm. There was a difference of almost 5 per cent in separable fat in the short-fed steers, none at all in the long-fed. This is worthy of note for two reasons: first, it is obvious that stilbestrol feeding does not shorten the time required to feed cattle to the desired degree of fatness. Therefore, stilbestrol-fed cattle must be marketed at heavier weights than non-stilbestrol-fed. During several weeks this past fall, the average weight of steers

TABLE IV

Data on Control and Stilbestrol-fed Steers, Beltsville, Md.

Item	Control	10 mg. stilbestrol/day	Control	10 mg. stilbestrol/day	30 mg. stilbestrol/day	60 mg. stilbestrol/day
No. of days on test	84	84	176	176	151	151
Initial wt., lb.	800	800	600	600	800	800
No. of steers	5	5	5	5	1	1
Av. daily gain, lb.	1.91	2.03	1.99	2.26	1.83	1.74
Live grade	Choice	Low choice	Low choice	Low choice	Top good	Good
Carcass grade	Low choice	Low choice	Low choice	Low choice	Good	Top commercial
Dressing, %	60.8	61.4	60.9	61.3	64.0	60.0
Separable fat, % (9, 10, 11 rib sample)	36.3	31.4	36.9	36.9	39.2	31.6
Water in round, %	73.3	73.6	—	—	—	—
Ether extract in round, %	3.9	4.0	—	—	—	—
Warner-Bratzler shear test, lb.	10.0	9.4	11.1	12.8	15.0	17.3
Liver, lb.	9.2	10.9	9.0	9.7	17.0	14.0
Pituitary, Gm.	2.0	2.4	1.8	2.1	4.2	3.6
Adrenal, Gm.	16.2	18.7	14.9	17.4	25.1	18.2
Thyroid, Gm.	20.6	20.1	14.2	13.0	28.7	21.3

TABLE V

Nitrogen Retention Trials with Steers

Period	Nitrogen retention	
	Control, Gm.	Treated, Gm.
7 days pretreatment	8.5	8.8
7 days ending treatment day 14	32.0	51.3
7 days ending treatment day 31	27.7	55.1

marketed exceeded the average weights of cattle marketed during the corresponding weeks last year by as much as 75 pounds per head. Was this caused by widespread stilbestrol feeding? The second point is the well-worn one that the housewife is perfectly content to accept leaner meat, even though we know that there is a real relationship between "quality" of cut and fatness of carcass.

The liver, the pituitary, and the adrenal show significant weight increases in the treated animals. We cannot yet evaluate these differences but they are real and reasonable. It is well established that stilbestrol is metabolized in the liver and it is just for that reason it is not, and probably cannot be, stored in the tissues of cattle fed in the permitted manner.

With respect to the pituitary and the adrenal, and briefly to the thyroid, too, for thyroid weight is no criterion of its activity, we must finally turn to the problem of how stilbestrol acts to produce its consistent and sometimes dramatic effect on increase in weight. Clegg and Cole[31] have presented an adequate working hypothesis, compatible with the facts we now know. In extensive experiments with 240 treated steers in eight trials, 100 heifers in four, 66 ewe lambs in two, and 51 wether lambs in two, using stilbestrol pellets, they found consistent increases in rate of gain. They, too, found that pituitaries and adrenals of treated animals were significantly heavier than those of controls. The adrenal hypertrophy was due to cortical enlargement. They conducted nitrogen retention trials with steers (table V). Obviously, the nitrogen retention of the treated steers was sharply greater than that of controls, as it must be in order to support more rapid gain without increase in rate of fat deposition.

Clegg and Cole state, "The fact that feed consumption is slightly increased but the economy of gain is greatly increased would point to the fact that protein anabolic processes are accelerated. Growth hormone and androgens are both effective in increasing nitrogen retention but our results would indicate that androgen is probably responsible for this effect in cattle and sheep. . . . That this is an indirect effect mediated by the pituitary is indicated by the occurrence of an androgenic response. The steer carcass has a similar appearance to the intact male. During the course of some of the trials, both steers and heifers developed a typical nymphomania stance, i.e., an elevated tailhead. Excessive riding was a characteristic behavior during the first few weeks after treatment. The animals behaved in a bull-like manner, i.e., they bellowed and pawed the ground."

In conclusion, may I point out that antibiotic feeding, hormone feeding, arsenical feeding, and the constant flow of new vitamins present to the research

worker a welter of problems on interrelationship. Braude and his associates[32] have made a major exploration in this field with equivocal results. Beeson and his associates[33] have made another. There will be a lot more work done before we can place the stilbenes, the tetracyclines, arsenic, the many vitamins, and the recipient animals' own hormones in optimal amounts absolutely, and relative to one another, to maximize rate of gain, feed efficiency, and control of product quality and composition.

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An Experimental Design for the Determination of Stilbestrol Residues in Steer Tissues

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If one attempts to prove the absence of a substance or to measure the amount of a substance that exists in very minute amounts, difficulties are encountered that are not present in the ordinary analytical method. The assay of stilbestrol residues in steer tissues presents such a problem.

The first question we must answer is, "Does the feeding of stilbestrol to steers result in added estrogenic activity to the edible tissues?" The second question is, "What is the sensitivity of the method?" That is to say, if no difference in the amount of estrogenic activity between tissues from control steers and treated steers is detected, what is the figure we can use when we say that "there is less than 'blank' amount of added estrogenic activity due to stilbestrol"?

We can answer the first question tentatively by feeding tissue from control steers to immature or castrated mice and comparing the uterine weight of the mice with the uterine weight of similar mice fed tissues from stilbestrol-treated steers. A significantly higher uterine weight in the latter group would indicate added estrogenic activity. By including tissue from normal steers as a control, we attempt to correct for the effect of any naturally occurring estrogenic activity.

In order to quantitate the amount of estrogenic activity found, we need to establish a standard curve by measuring the uterine weight response to known amounts of stilbestrol.

In our experiments, we fed tissue diets to immature mice for seven days beginning with their twenty-second day of life. On the eighth day of the experiment, the animals were sacrificed and their uteri weighed without fixation. The steer tissues, stored in the frozen state, were thawed and ground three times with standard laboratory chow in the proportion of 100 parts of tissue to 10 parts of chow. The various concentrations of stilbestrol were added to the mouse diet in the form of an alcoholic solution so adjusted that 100 Gm. of diet received 1 ml. of alcohol. After stirring thoroughly with an ordinary kitchen mixer, the diets were made up into 6 Gm. patties and refrozen. Each animal received one 6 Gm. patty per day. This amount of diet was all consumed and was nutritionally sufficient to produce some growth. Individual records of initial and final body weights and feed consumption were kept.

Standard curves have been established and used in various ways. The curve should be established by adding various dosage levels of stilbestrol to the particular tissue under test, and one should be included with each assay. The results from

TABLE I

Average Uterine Weights of Groups of 5 to 6 Immature Mice Fed Various Control Tissues for Seven Days

Composition of diet	Number of experiments	Mean av. uterine wt., mg.	Range of averages, mg.
Chow 100	2	12.6	12.3–12.9
Chow 10, lean 100	13	7.4	5.6– 9.8
Chow 10, liver 100	15	7.9	6.4–10.4
Chow 10, kidney 100	5	7.1	6.2– 8.0
Chow 10, offal 100	2	4.0	4.0– 4.1
Chow 67, fat 33	5	17.5	11.7–32.9

feeding the stilbestrol-treated tissue to a single group of mice can then be estimated by reading off this single standard curve prepared from control tissue. Statisticians and bioassayists have found it advantageous to prepare two curves, one for the known or control tissue and one for the unknown or treated tissue. The two curve method allows them to make certain calculations for the validity of the assay. Some experimenters have established the standard curve by adding the various levels of stilbestrol to the regular laboratory mouse ration and have used a previously established standard curve for evaluating the results. We recommend that two curves be established with each assay, one for the control tissue and one for the stilbestrol-treated tissue.

Table I illustrates some of the reasons for this. Column one shows the various tissue diets we have tested, and column three shows the mean of the average uterine weights obtained in the number of experiments shown in column two. Note that the mean average uterine weights varied from 4.0 mg. for animals fed offal, to 17.5 mg. for those fed fat. The value for chow is 12.6 mg. and significantly greater than for lean meat, liver, or kidney. We do not believe these differences represent differences in the level of estrogenic activity in the various diets, and we are not sure it is entirely a matter of nutrition, since all of the animals gained weight. The range indicates the amount of variation found. These data point up our contention that a standard curve should be established for each tissue and one included for each assay.

Table II further illustrates the effect of diet composition on the uterine weight. Here the ratio of fat to lean was changed in the first four diets, and the fifth diet

TABLE II

Effect of Composition of Diet on the Average Uterine Weight of Groups of 5 to 6 Immature Mice Fed for Seven Days

Composition of diet			Av. uterine wt., mg.	Av. final body wt., Gm.	Uterine wt. (mg.) / body wt. (Gm.)
Lean	Fat	Chow			
100	0	10	8.7	10.0	0.87
90	10	10	14.2	13.3	1.07
80	20	10	21.9	13.8	1.59
70	30	10	21.4	15.2	1.41
0	33	67	17.1	14.0	1.22

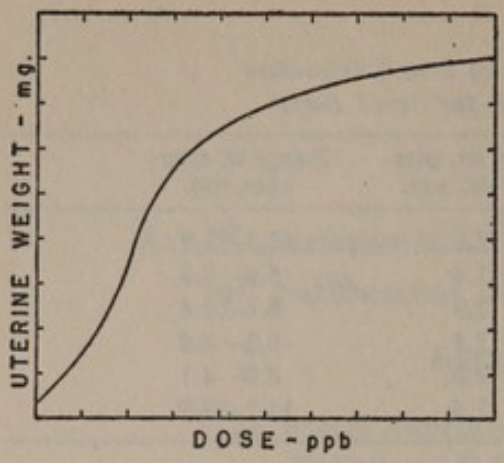

FIG. 1. Typical sigmoid dosage-response curve for mouse uterine weight to estrogens.

was included for comparison. Note that the uterine weight, as well as the body weight, tends to increase with increasing amounts of fat in the diet. Since others have used the uterine weight to body weight ratio for calculation, we have included it for comparison. We have not used it, since our data indicate that it is not a linear relationship. To avoid lengthy calculations of covariance to correct for this relationship, we have divided our mice among the various groups so that each group will have an equal average initial body weight.

Figure I shows the typical sigmoid dosage-response curve for mouse uterine weight to estrogens. One would ordinarily select a dosage so that the response would fall on the steepest part of the curve. Here, transformation to log dose response will usually result in a straight line.

Since we are attempting to measure amounts of stilbestrol in tissue at near zero levels, we are on a portion of the curve that is very flat. In order to get responses that are on a more favorable part of the curve, we can add stilbestrol to the treated tissue in the same dosage levels that we have added stilbestrol to the control tissues to establish the standard curve. We thus have two curves, one for the standard and one for the unknown. The distance between the two curves will be a measure of added estrogen in the tissue.

Figure 2 shows the results of a typical assay by this method. The upper two curves represent the uterine weight response of control and treated tissue to which

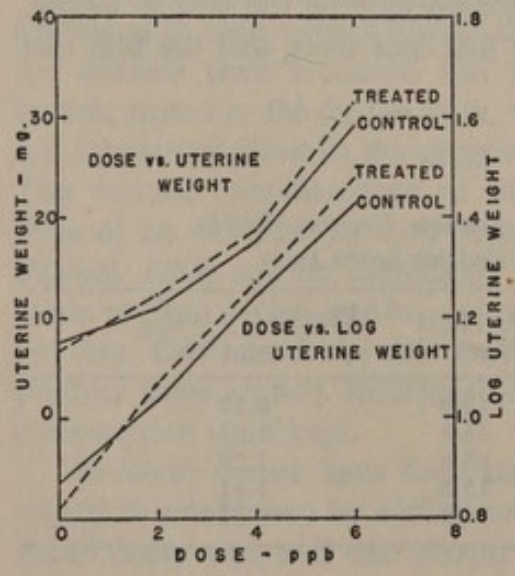

FIG. 2. Results of a typical assay plotted two ways.

doses of 0, 2, 4, and 6 parts per billion of stilbestrol have been added. Note that there is definite curvature. The lower two curves show the same data plotted as log of the uterine weight versus the dose. The curves are linear and parallel, and there is no significant difference between the control and the treated group. In this particular assay, the sensitivity of the method is calculated as 0.5 part per billion.

It should be pointed out that, if we were on a portion of the sigmoid curve where it is necessary to plot log dose versus response in order to achieve linearity, the method could not be used. If any estrogenic activity were present in the tissue from stilbestrol-treated steers, the curves could not be both linear and parallel.

One further word of caution should be mentioned. The curves should be prepared with the actual estrogen being tested for. It is well known that the slope of the dosage response curves for various estrogens differ.

SUMMARY

An experimental design has been described for the assay of stilbestrol residues in steer tissues in which known doses of stilbestrol are added to both the control and the stilbestrol-fed tissues. The design lends itself to a statistical analysis of the validity of the assay and an estimation of the sensitivity of the method.

Antibiotics in Animal Feeds for Therapy

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The use of antibiotics in feeds for therapy is the most economical method for the mass treatment of flocks and herds. This method of therapy is used widely today because of its effectiveness and relative simplicity. Since the meaning of the term "therapy" as it applies to the use of medicated feeds may differ somewhat from the generally understood concept, this term will be defined before proceeding further. Therapy with medicated feeds is the treatment of an entire flock or herd where disease is evident in varying degrees in some of the animals rather than the singling out of individual animals for treatment.

In the past there has been a tendency to express the antibiotic effect in nutritional terms because the effects in most of the experimental work were measured in terms of weight gains and feed efficiency. Actually, a depression in growth rate and feed efficiency can be manifestations of disease just as classical clinical symptoms are. In fact, impaired growth rate and feed conversion are the most sensitive indicators of the presence of disease and frequently the only indicators of the presence of subclinical disease, or the incubation and recovery stages of clinical diseases. Since the principal or sole effect of antibiotics is on microorganisms, it would seem logical to conclude that both the correction of clinical symptoms and the improvement in growth and feed efficiency are therapeutic effects resulting from the feeding of antibiotics. Thus, what in the past was referred to as the so-called nutritional effect of antibiotics, was in reality the correction of the growth-inhibiting effects of disease.

THE BASIS FOR SELECTING AN ANTIBIOTIC FOR USE IN FEEDS

Of the many different antibiotics known today, a number are available for use as feed supplements. Frequently, these are considered to be interchangeable for feed usage; however, this is no more correct than considering other classes of drugs as interchangeable for therapeutic purposes. All antibiotics do not possess the same degree of effectiveness; therefore, in order to ensure good results, only the antibiotic best suited for each individual problem should be used. A list of the characteristics of the theoretically ideal antibotic is presented here as a measure of comparison in selecting the most effective antibiotic. The ideal antibiotic would be: (1) active against a wide range of disease organisms; (2) safe at high concentrations; (3) palatable at high concentrations; (4) readily absorbed through the intestinal wall and well distributed systemically; (5) retained in the tissues

long enough to be effective; (6) stable both in the feed and in tissues, and (7) one to which organisms do not readily develop resistance.

Probably no antibiotic will ever meet all of these specifications. This list is presented to set a standard and to illustrate first the importance of evaluating each antibiotic on the basis of its chemotherapeutic properties and second to point up the value of comparing the different antibiotics before selecting one for practical use. The fact then becomes more evident that each antibiotic has certain characteristics that determine its usefulness as a feed additive under different conditions. Some antibiotics are more stable, and some may be less toxic. Disease organisms may develop resistant strains quickly against one antibiotic, but not against another. Or, one antibiotic may lack effectiveness because it is not absorbed. For example, an antibiotic that is not absorbed systemically can hardly be expected to have any marked effect on a case of bacterial pneumonia; yet this same antibiotic could be at least partially effective against a case of bacterial enteritis. Because most disease conditions are not single entities, a broad-spectrum antibiotic that is readily absorbed is usually the best so that not only the clinical disease, but also the secondary, or subclinical, infections will be controlled. In addition, diseases vary in their susceptibility to antibiotics; therefore, it is necessary to know exactly what level of antibiotic is needed for successful treatment. Because of possible toxicity or palatability problems, higher levels of certain antibiotics cannot always be used. Numerous other examples could be given, but, from those already cited, it is obvious that successful treatment is dependent upon the careful selection of the right level of the right antibiotic, as determined by the individual disease problem.

RECENT EXPERIMENTS ILLUSTRATING THE USE OF ANTIBIOTICS IN FEEDS FOR DISEASE THERAPY

Therapy of Specific Diseases. TURKEY ORNITHOSIS. Davis and Delaplane[1] at A. & M. College of Texas studied the effect of antibiotics in feeds for the control of turkey ornithosis. In early trials they found mortality could be prevented in 3 week old poults if their feed contained 100 Gm. of chlortetracycline*/ton.

A later trial was designed to determine whether or not the disease could be completely eliminated from an infected flock. Levels of 0, 10, 100, 200, and 400 Gm. of chlortetracycline per ton of feed were fed to poults that had been artificially infected with ornithosis virus. At the end of a two week feeding period, all birds were placed on an antibiotic-free ration and a few days later were sacrificed for virus isolation studies. The results, as shown in table I, indicate that: (1) 10 Gm. of chlortetracycline per ton of feed reduced mortality; (2) 100 Gm. of chlortetracycline per ton eliminated mortality; (3) 200 Gm. of chlortetracycline per ton eliminated symptoms of the disease as well as mortality; and (4) 400 Gm. of chlortetracycline per ton eliminated virus recovery, disease symptoms, lesions, and mortality. Thus, to prevent the spread of ornithosis, a level of 400 Gm. of chlortetracycline per ton of feed may be necessary; however, the reduction in virus titer seen with 200 Gm. may also be below the natural transmission threshold. If control need not be this complete, even lower levels probably would suffice.

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* The trade name of American Cyanamid Co. for chlortetracycline is Aureomycin.

TABLE I

*The Use of Chlortetracycline in Poults Infected with Ornithosis**

Gm. chlortetracycline/ton of feed	No. birds	Symptoms†	Lesions†	Mortality, %	Virus recovery†
None	50	+	+	94	+ all attempts
10	25	+	+	64	+ all attempts
100	50	+	+	0	+ 68%
200	50	−	+	0	+ 8%‡
400	25	−	−	0	−‡

* Adapted from Davis and Delaplane.[1]

† + Present or positive; − absent or negative.

‡ Complement fixation inhibition at end of test, all negative.

This variation in degree of control exemplifies the results that have been experienced with many other diseases treated with graded antibiotic levels and also emphasizes the fact that the clinician must use utmost care in selecting not only the right antibiotic, but also the dosage level adequate for control of the particular conditions in an individual herd or flock.

Enterotoxemia in Sheep. The work on the control of enterotoxemia in sheep carried out by Johnson and his associates[3] is cited as another practical example of the treatment of a specific disease by mass feeding of an antibiotic.

Feeder lambs were divided into eight groups of approximately 100 animals each at the time they were brought into the feed lot. Four different feeding regimens (table II) were started immediately with two replicate groups on each regimen. Feeding was continued for a 48 day period. Two asymptomatic deaths occurred in the first 21 day period. The 42 deaths that occurred in the period from 21 to 49 days were all cases of enterotoxemia confirmed by isolation of *Clostridium perfringens,* identification of the toxin, and the classical pathology. These deaths all occurred in groups 1 and 4, which either received no antibiotic at all or none after the twenty-first day. No deaths occurred in groups 2 and 3, which received the antibiotic for the entire 48 day period.

TABLE II

*A Progress Report on the Use of Chlortetracycline in Sheep Feeds for the Control of Enterotoxemia**

Feeding regimen no.	Treatment	No. animals	Mortality 0–21 days	Mortality 21–49 days
1	No chlortetracycline	199	2 deaths	12 deaths
2	Continuous chlortetracycline: 50 Gm./ton for 21 days, 50 Gm./ton for 27 days	199	0	0
3	Continuous chlortetracycline: 20 Gm./ton for 48 days	200	0	0
4	Intermittent chlortetracycline: 50 Gm./ton for 21 days, no chlortetracycline for last 27 days	200	0	30 deaths

* Adapted from Johnson et al.[3]

TABLE III

*The Effect of Feeding Chlortetracycline to Pigs with Atrophic Rhinitis; Comparison of Response of Light and Heavy Weight Pigs**

	Chlortetracycline, Gm./ton			
	Light groups		Heavy groups	
	0	50	0	50
No. pigs started	25	25	25	25
No. days on trial	90	90	60	60
Av. initial weight (lb.)	78	72	133	131
No. pigs lost or removed	1	0	0	2
Av. final weight	202	223	246	253
Av. daily gain	1.37	1.66	1.88	2.04
Feed per 100 lb. gain (lb.)	415	390	433	412

* Adapted from Gouge et al.[2]

Therapy of Secondary Infections with Antibiotic Feeds. In addition to having a direct effect on certain specific diseases, some antibiotics are useful in feeds because of their effect on secondary bacterial invaders that almost invariably accompany specific diseases. This effect on secondary invaders is of value whether the antibiotic is or is not active against the agent of the specific disease.

Secondary Infections Accompanying Atrophic Rhinitis. As an example, chlortetracycline has shown no effect in altering the specific pathology of atrophic rhinitis in pigs, yet in a field trial carried out by Gouge and his associates[2] swine naturally infected with atrophic rhinitis were found to gain normally when given a feed containing chlortetracycline, while a similar group of infected pigs fed the basal ration made only poor and inefficient weight gains (table III). The improved growth of the antibiotic-fed animals was probably due to the control of the secondary bacterial infections that usually accompany atrophic rhinitis, namely, chronic pneumonic complications and middle ear infections. Thus, it seems that the primary cause of economic losses from outbreaks of atrophic rhinitis is not the specific disease itself, but the secondary infections accompanying it.

DISCUSSION

The success of an antibiotic feeding program has been found to be dependent upon careful selection of the right antibiotic at the right level for an adequate treatment period, as determined by the individual disease problem. The work by Davis and Delaplane[1] on turkey ornithosis was used to illustrate the variations in results that follow the use of different levels of the same antibiotic; that is, death losses in affected birds could be eliminated by the use of a feed containing 100 Gm. of chlortetracycline per ton, but, in order to eliminate the infection completely in a flock, a level of 400 Gm./ton was required. The work by Johnson and his co-workers[3] on sheep enterotoxemia showed the importance of continuous feed therapy for effective control against a specific clinical disease.

Since the chemotherapeutic properties of antibiotics vary widely, these drugs cannot be used interchangeably. Numerous reports in the literature show the

tremendous variations in activity, safety, and stability that exist even between antibiotics that are closely related chemically. Experience has shown that usually the antibiotic of choice is one active against a wide spectrum of pathogenic organisms and one well absorbed systemically so that the medication will be effective against an entire disease complex, not just a single entity.

As has already been pointed out, disease in a herd may be either apparent (clinical), or unapparent (subclinical). One stage so consistently overlaps the other that a sharp differentiation between them cannot be drawn; therefore, if antibiotic feeding is to be effective, all stages of disease in the herd or flock must be considered. Mass therapy with antibotics is a combination of treatment of the visibly sick and prevention in the apparently unaffected individuals. But, because the basic principle behind the use of antibiotics in feed is that they act primarily if not solely against microorganisms, feeding antibiotics to a herd is actually a therapeutic measure.

In certain cases in which disease is acute, individual therapy may be required if an animal is to be saved. Sick animals that have gone off feed decrease the antibiotic intake proportionate to the decrease in feed intake and cut down on the effectiveness of this method of treatment. In cases in which the value of the animal exceeds the additional cost of drug and labor required for individual therapy, this method can be used in conjunction with mass feed therapy of the entire herd. By using this combined method, the exposed animals as well as the clinically sick can be treated at one time and disease transmission can be prevented.

The primary cause of economic losses in a herd or flock often is not due to the effects of the clinical disease itself but to the secondary infections accompanying it, which cut down on feed efficiency and rate of growth. In some instances antibiotics may have no effect in altering the pathology of a specific clinical disease, as in the case of the trial carried out by Gouge and his associates[2] with swine affected with atrophic rhinitis; yet, feeding an antibiotic may make it possible for affected animals to gain normally, as did the pigs fed chlortetracycline in this swine trial. There are times when disease is present in a flock or herd without showing evidence of any classical symptoms at all. The only indication of the presence of disease is the economic loss caused by poor feed conversion and poor weight gains. In such instances the continuous feeding of antibiotics can cut down or eliminate this problem and thereby improve the performance of the animals.

Thus, therapeutic antibiotic feeds make savings possible in the following ways: (1) by lowering or eliminating death and morbidity losses in visibly sick animals, (2) by improving weight gains and feed efficiency, (3) by lessening the recovery period needed once the disease is brought under control, and (4) by minimizing the further spread of the infection in the herd or flock.

CONCLUSION

The use of antibiotics in feeds for therapy is generally the most economical and practical way, on a herd basis, to treat most diseases of domestic animals. In cases in which individual therapy is required, some other method of treatment, such as injection, may be preferable; in these cases, individual therapy is best used in conjunction with continuous mass feed therapy so that not only the clinical stages but also other stages of disease that may be present in the flock or herd can

be treated and thus disease transmission to unaffected animals in the group may be prevented.

Since antibiotics act primarily, if not solely, on disease organisms, what in the past has been referred to as the so-called nutritional effect of antibiotics is now considered to be an effect brought about by the correction of the growth-inhibiting effects of disease. Because antibiotics are not interchangeable and since the effectiveness of an antibiotic feed is dependent mainly on the individual antibiotic selected (assuming an adequate level is used), the pharmacologic and chemotherapeutic properties of each one must be carefully considered in relation to the particular disease problem before a final choice is made. The practice of considering antibiotics of equal therapeutic value can only lead to inconsistent or ineffective results. Disease is usually not caused by a single entity but by a complex of pathogens; thus, the most effective antibotic usually is one that has a wide range of antimicrobial activity and one that is well absorbed systemically

The most serious losses dollarwise occur during the incubation and convalescent periods of frank disease and during periods of subclinical disease that may or may not be present coincidentally with acute disease outbreaks. In most cases, the continuous feeding of a therapeutic antibiotic ration to the entire herd or flock will improve performance by controlling the disease problem, by eliminating death losses, and by bringing the animals back into more efficient production.

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Antibiotics in Growth Promotion of Livestock

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A large volume of research data has been accumulated during the past few years demonstrating that antibiotics have definite growth-promoting properties for livestock and poultry. To benefit livestock and poultry, antibiotics have been used in three ways: (1) When utilized in veterinary medicine, they provide an effective treatment for specific infectious diseases. (2) When fed at relatively low levels in good rations to apparently normal animals, they increase rate of growth and efficiency of feed conversion. The degree of improvement varies with the species and age of animal, the type of antibiotic, and environmental factors. (3) When fed at relatively high levels, antibiotics greatly enhance livestock production by diminishing mortality and production losses and retarded feed consumption and growth due to ill-defined infections as well as those of a more specific nature.

My remarks concern the phase described as the growth promotion of animals with so-called "nutritional" levels of antibiotics. In connection with each species, there will be considered, insofar as substantial data are available, the relative effectiveness of different antibiotics, the optimum period for feeding antibiotics, and effect on carcass quality. In connection with the use of antibiotics in ruminants, consideration will be given to effects on rumen function. I shall also deal briefly with possible mechanisms through which antibiotics promote growth.

CHICKENS

Existing knowledge indicates considerable difference among various antibiotics in their effectiveness for promoting chick growth. For this purpose, penicillin has advantages over bacitracin, chlortetracycline,* and oxytetracycline,† as shown by table I, summarizing many trials by college and commercial laboratories.

TURKEYS

A picture similar to that of chickens has been obtained in the comparison of the data of many antibiotic growth tests with growing turkey poults (table II).

According to Stokstad,[1] the majority of the observed growth increases in turkeys as a result of antibiotic feeding either at 4 or 8 weeks falls between 10 and 30 per cent. Feed efficiency is enhanced from 5 to 10 per cent.[2]

* The trade name of American Cyanamid Co. for chlortetracycline is Aureomycin.
† The trade name of Chas. Pfizer & Co. for oxytetracycline is Terramycin.

TABLE I

Effect of Antibiotics on Chick Growth, as Per Cent of Growth without Antibiotic

	Grams antibiotic per ton feed							
	0.6	1	1.2	2	3	5	6	10
Penicillin	114		117		122		121	
Oxytetracycline		108.5		111		112		114
Chlortetracycline				109		113		115
Bacitracin				108.5		116		116

Stokstad adds that growth responses of 7 to 11 per cent have been observed in goslings fed penicillin and responses also have been obtained with chlortetracycline, penicillin, streptomycin, and oxytetracycline. In one reported study with ducks, there was no growth increase from the addition of antibiotics. Limited trials with pheasants, according to Heuser,[3] showed "a larger increase in weight than with chickens," while ducks "showed a smaller gain" as a result of antibiotic feeding.

In general, it is the consensus of experience that, in applying antibiotics to broiler and turkey raising, one should include them in the ration throughout the entire growing period. If the antibiotics are fed for only a part of the growth period, there is negligible carry-over of growth effect and before long a regression to the nonsupplemented rate.

SWINE

Extensive feeding tests have demonstrated that antibiotics improve the growth rate of apparently healthy pigs 10 to 20 per cent. The growth-promoting effect is most marked in younger pigs and decreases as they get older. Feed efficiency is also improved up to 10 per cent. In unthrifty and "runt" pigs, the growth rate has been improved as much as 100 to 200 per cent. In addition to improving growth rate, antibiotics also combat various enteric diseases in pigs.

A study of the literature up to 1955, with careful effort to utilize only data of experiments without complicating factors of wide diversities in rations and disease level and with attention to equivalence of levels of supplementation, shows the relative growth-promoting efficiencies given in table III.

TABLE II

Effect of Antibiotics on Poult Growth, as Per Cent of Growth without Antibiotic

	Grams antibiotic per ton feed						
	1	1.2	2	3	5	6	10
Penicillin		128		130		127	
Oxytetracycline	108		111		119		123
Chlortetracycline	112		110		123		118
Bacitracin	111		109		123		128

TABLE III

*Comparative Growth-promoting Effect of Antibiotics**

	Growth index†
Chlortetracycline	116.5
Penicillin	111.4
Oxytetracycline	116.7

* Based on 95 literature reports, 166 observations; Chas. Pfizer & Co., Inc. Agricultural Technical Bulletin No. 23.
† Growth of controls = 100.

A comparative review of antibiotics in swine nutrition, by Braude et al[4] illustrates the necessity for attention to variables such as basal ration, health of animals, and level of supplementation in evaluating studies. In their review, which covers literature from the inception of antibiotic feeding up to about the end of 1952, these authors recognized the heterogeneity of the data and divided their figures into three groups as shown in table IV. One group constituted data of the Florida Agricultural Experiment Station where in many experiments there was used a corn-peanut meal ration found to be nutritionally inadequate for growing pigs without antibiotic supplementation. Another group comprised mainly trials from the Hormel Institute. In these the control animals suffered severe digestive disorders.

The data in the first two columns show enormous advantages from several of the antibiotics, but these, in view of the factors pointed out, are more truly a reflection of a therapeutic effect than of practical value as a nutritional supplement.

From their data, it is possible to compute that the control animals in these two groups gained at a rate of only .62 lb./day. In contrast is a third group, designated as "others," in which the rate of gain of the controls was 1.27 lb./day.

Moreover, in the Florida group, only 15 per cent of the animals received a nutritional level of less than 25 Gm. of antibiotic per ton of feed, and in the Hormel group 55 per cent received less than this level, whereas, in the "others," 86 per cent received less than 25 Gm. per ton.

In other words, the "others" represent more nearly typical conditions of health and well-being of pigs and a more typically "nutritional" level of feeding, and

TABLE IV

*Comparative Growth-promoting Effect of Antibiotics**

	Growth indexes		
	Florida	Hormel	Others
Chlortetracycline	179	161	115
Oxytetracycline	190	152	118
Streptomycin	128	257	109
Penicillin	97	143	110
Bacitracin	100	—	111
Chloramphenicol	106	117	98
Gain of controls, lb./day	0.62	0.62	1.27
% < 25 Gm. antibiotic/ton	15	55	86

* Based on data from Braude et al.[4]

TABLE V

Antibiotic Withdrawal — Growing Fattening Swine

	Control	Chlortetracycline		Oxytetracycline	
		To 125 lb.	To 225 lb.	To 125 lb.	To 225 lb.
No. animals	15	15	15	15	14
Days on feed	111	97	94	102	94
Initial weight, lb.	49.7	50.5	49.5	50.5	51.7
Final weight, lb.	228.3	225.2	227.5	225.9	225.2
Daily gain, lb.	1.61	1.80	1.89	1.72	1.84
Lb. feed/lb. gain	3.70	3.53	3.43	3.56	3.43

hence present more suitable conditions for comparison of antibiotics. The growth index figures under "others" show that chlortetracycline and oxytetracycline are about equal and enhance growth of swine to a distinctly greater degree than streptomycin, penicillin, or bacitracin.

It is the general practice to continue antibiotic feeding throughout the entire growing-fattening period. The data presented in table V, based upon a report by Wilson,[5] illustrate that most efficient growth does not result if antibiotics are discontinued before market weight is attained.

The figures for "days on feed" demonstrate that earlier marketing is possible with antibiotic supplementation of swine rations.

CARCASS QUALITY

The effect of antibiotic feeding upon carcass quality has received close attention in connection with the feeding of swine. Considerable study has been given to this, both in the United States and abroad.

In Denmark, the extensive observations of Clausen[6] have shown no effect from antibiotics under conditions of restricted feeding as practiced in Denmark. Physical separations and chemical analyses also were conducted and results indicate no change in composition of carcass, so that, under these conditions, antibiotic supplementation brings about a true enhancement of meat production.

In swine fed according to appetite (ad libitum), the literature is somewhat confusing concerning effects of antibiotics on carcass quality, but there appears to be a preponderance of studies, in the American literature, at least, showing no decrease in carcass quality (see table VI).

TABLE VI

*Effect of Antibiotic Feeding on Carcass Quality of Swine**

	Control	Antibiotic-fed
Growth		
Average daily gain, lb.	1.40	1.52
Increase, per cent	—	9
Carcass data		
Dressing per cent	75.7	75.9
Depth backfat, inches	1.73	1.78
Carcass length, inches	29.04	29.30
Number of observations	186	269

* Based on data in the literature.

TABLE VII

Per Cent Cellulose Digested of the Amount Present at the Beginning of 24 and 36 Hour Incubation Periods by Rumen Bacteria in Vitro as Influenced by Eight Antibiotics — Artificial Rumen

Antibiotic, μg. per ml.	Penicillin G sodium		Dihydro-strepto-mycin		Poly-myxin B sulfate		Chloram-phenicol		Oxytetra-cycline		Bacitracin		Chlortetra-cycline		Carbomycin	
	24 hr.	36 hr.	24 hr.	36 hr.	24 hr.	36 hr.	24 hr.	36 hr.	24 hr.	36 hr.	24 hr.	36 hr.	24 hr.	36 hr.	24 hr.	36 hr.
0	47	91	47	91	47	91	47	91	47	91	—	91	—	91	47	—
3.66	2	7	38	77	44	88	51	90	41	91	—	90	—	88	8	—
7.33	4	0	31	79	48	81	50	94	39	92	—	84	—	86	1	—
14.66	5	10	21	62	45	90	47	88	30	87	—	61	—	90	2	—
29.32	2	—	2	—	42	—	22	—	11	—	—	—	—	—	—	—

TABLE VIII

Per Cent Digestion of Cellulose by Oxytetracycline-treated Cows Determined by the Artificial Rumen Technique

Animal no.	Oxytetracycline	Pretest period, %			Day of treatment				Days after treatment				
					1st day, %	2nd day, %	5th day, %	8th day, %	1st day, %	3rd day, %	4th day, %	6th day, %	9th day, %
1	None	66	69	72	60	58	—	47	—	—	56	54	—
2	50 ppm in feed	50	44	68	59	44	—	54	—	—	51	64	—
3	200 ppm in feed	64	58	68	25	49	—	69	—	—	51	63	—
4	2.5 Gm. daily	62	60	66	26	34	48	—	56	—	—	—	47
5	5 Gm. daily	49	41	42	15	11	24	—	54	57	—	—	52

The economic benefits of supplementing swine rations with antibiotic have been computed by the Iowa Agricultural Experiment Station[7] from 31 experiments comprising 1814 pigs. Averages are expressed as for a 20 sow herd. The manpower saving per litter raised is equivalent to six 8 hour days; the feed saving is 6240 pounds and the net dollar saving, allowing for cost of antibiotic, is $156, equivalent to $1.20 more profit per pig.

RUMINANTS

Effect of Antibiotic Feeding on Rumen Function. In connection with the feeding of antibiotics to cattle and sheep, attention has focused on possible effects of these agents upon the microbial fermentation processes that are so essential to the nutrition of ruminant animals. This interest was stimulated especially by reports of Bell et al[8, 9] and Colby et al[10] that feeding certain antibiotics to cattle and sheep depressed feed intake and brought other adverse effects.

Subsequently, many reports on antibiotic feeding trials with ruminants have appeared and in nearly a score of these attention has been given to the effect of these treatments on digestion, cellulolytic power of the rumen, composition of rumen microflora, and other possible indexes to the effect of antibiotics on rumen function.

To illustrate these effects, there may be cited the extensive series of experiments by Hardie et al[11, 12] who compared the action of eight antibiotics upon cellulose digestion by steers, using the artificial rumen technique. They also traced the time relationships of changes in cellulolytic power as affected by feeding oxytetracycline.

It will be seen from table VII that polymyxin B sulfate and chloramphenicol had little effect on cellulolytic activity. Bacitracin and dihydrostreptomycin showed some inhibition of cellulose utilization, which increased as concentration of the antibiotic increased. At 36 hours incubation no significant inhibition was seen with either chlortetracycline or oxytetracycline. By the methods employed, carbomycin and penicillin showed marked inhibition of cellulose digestion.

An indication of the effect of time lapse is shown in a more detailed study of oxytetracycline included in the same report (table IX).

TABLE IX

Per Cent Cellulose Digested of the Amount Present at the Beginning of 24 and 48 Hour Incubation Periods by Rumen Bacteria in Vitro as Influenced by Oxytetracycline

Oxytetracycline, μg. per ml.	Approximate equivalent dosage, ppm of feed	24 hours' incubation, %	48 hours' incubation, %
None	0	68	89
7.33	50	27	93
14.66	100	10	84
36.66	250	8	44
73.32	500	2	38

Here again the degree of depression was proportional to the concentration of antibiotic, but within 48 hours the cellulolytic activity of all but the two highest levels of oxytetracycline was equivalent to that of the flask containing no antibiotic. In this table is given also a column showing concentrations used in terms of equivalent concentrations per ton of complete feed for a theoretical bovine.

This study was extended to include a feeding trial with 5 mature lactating cows of which 1 was a control and the other 4 received the various levels of oxytetracycline shown in table VIII. The 50 and 200 ppm levels were included in the feed. The 2.5 and 5 Gm. levels were administered by capsule, daily. This study included a pretest period of one week during which three rumen samples were taken by stomach tube. The test period lasted eight days and four samples were taken. The samplings were continued during a nine day period following treatment.

This test shows clearly that, in these mature cows, as the period of treatment is extended, the depressing effect of oxytetracycline on cellulolytic power rapidly disappeared, even though the level of antibiotic intake was fairly constant throughout the test period.

Similar data covering a time range have been obtained in calves and steers. A study by Hardie et al[12] was designed to obtain information on the effect of antibiotic feeding upon the onset of normal rumen function, the effect of withdrawal of antibiotic after it had been in the ration for some weeks, and the effect of introducing antibiotic feeding subsequent to the date that normal rumen activity would have commenced.

In this study, there were two groups of calves, of which one was a control and the other received 30 Gm. of oxytetracycline per ton of feed. At the end of 12 weeks both lots were divided. One half of the control group continued as such, and the other half began to receive the antibiotic (30 Gm./ton) at the thirteenth week. One half of the supplemented group continued to receive the antibiotic and the other half reverted to the unsupplemented ration.

The first rumen contents sample was taken near the end of the 0 to 12 week period, for during most of that interval the calves presumably were essentially monogastric animals. Eight more samples were taken during the 13 to 27 week period when rumen activity should be increasing rapidly.

Their figures show that, over the period studied, there was a gradual increase in the ability to digest cellulose, indicating no interference with the onset and continuation of rumen function, with the exception of some slight retardation in the group in which antibiotic feeding was introduced at the thirteenth week.

In this experiment, rates of gain of all groups that had received the antibiotic at any time were superior to that of the group that remained throughout as a control. Feed efficiencies also were superior in the animals that received the antibiotic at any time throughout all or part of the 27 weeks.

Steers were given two levels of oxytetracycline on high- and low-roughage rations. Rumen samples were withdrawn by stomach tube and tested for cellulolytic capacity by the artificial rumen, with results shown in table X.

This again demonstrates a short initial depression in cellulolytic capacity on both types of rations, but the effect rapidly wears off, even though the antibiotic feeding is continued.

There now have been published approximately a score of studies, mostly with chlortetracycline and oxytetracycline, of the effect of antibiotics on rumen cellulo-

TABLE X

Effect of Oxytetracycline on Rumen Function in Steers
Fiber Digestion — Per Cent

Day	High roughage, 9 steers			Low roughage, 9 steers		
		Oxytetracycline			Oxytetracycline	
	Control	75 mg./day	150 mg./day	Control	75 mg./day	150 mg./day
−1	40.9	44.3	45.3	40.5	34.9	39.2
1	38.8	17.6	3.4	36.9	20.8	17.6
2	34.5	26.6	32.9	32.7	40.1	44.5
3	43.3	38.4	40.8	44.4	45.8	45.2
4	37.5	34.9	32.1	40.9	39.7	41.9
7	56.6	55.9	49.1	61.8	63.6	67.2
8	44.7	43.0	44.4	43.8	55.4	60.9
14	50.7	55.0	59.2	50.8	52.0	56.1

lytic capacity, on digestibility, and on other facets of gastrointestinal activity of ruminants. While there is some divergence, the majority of findings appear to be in agreement with the results set forth in detail for oxytetracycline, namely, that any adverse effect on appetite and ration digestibility is only transient and that the ruminant animal readily becomes adapted to the presence of certain antibiotics in the ration.

Growth of Calves. The number of growth studies with antibiotics in calves is too great for individual citation in a paper of this scope. Owen et al[13] recently reported a comparison of several antibiotics and have related their findings to earlier results. They found the following enhancement of growth from antibiotics expressed as per cent of growth of control: Experiment I: oxytetracycline, 155; chlortetracycline, 151; penicillin, 86. Experiment II: oxytetracycline, 135; bacitracin, 126; chloramphenicol, 111. ". . . Efficiency of feed utilization was improved significantly ($P = 0.01$) in Experiment I by Aureomycin and Terramycin as compared with the penicillin and control groups. Comparison of all treated groups in Experiment II with the controls also suggested ($P = 0.09$) an improvement in efficiency of feed utilization."[13]

The authors note that the growth promotion from chlortetracycline and oxytetracycline is in accord with previous work and that the ineffectiveness of chloramphenicol corroborates, in general, observations with other species; and they cite work of three other sources that agree with their findings that penicillin is ineffective.

In a recent review[14] that cites 130 references, the benefits of antibiotic feeding to calves are summarized as increased growth rate; lower incidence of scours; increased feed consumption, particularly of concentrates, at an earlier age; improvement in feed efficiency as measured by pounds of feed required per pound of gain; and improvement in the calves' well-being. The enhancements in growth rate during the first 16 weeks of age have ranged from 10 to 30 per cent, although in a few studies no growth promotion has occurred. This review emphasizes also the importance of the scours-preventing effect of antibiotic feeding for young dairy calves.

TABLE XI

Oxytetracycline for Beef Cattle, 50 to 150 mg. per Head Daily

	Controls	Oxytetracycline	Per cent improv.
Summary of Growth and Feed Efficiency			
Growth			
No. trials	17	17	—
Av. daily gain, lb.	2.12	2.28	8
Feed Efficiency			
No. trials	15	15	—
Av. lb. feed/lb. gain	12.41	11.56	8
Summary of Carcass Quality Data			
No. trials	10	10	
Av. score*	9.27	9.32	
Av. dressing %	60.3	60.8	
Summary of Economic Data			
No. trials	17	17	—
Av. cost/lb. gain, $	0.231	0.219	5
No. trials	13	13	
Av. incr. ret./head	—	—	$3.44

* Prime: 15, 14, 13; choice: 12, 11, 10; good: 9, 8, 7.

Growth of Beef Cattle. In the previously mentioned digestion trials[8, 9] showing some adverse effect from antibiotics when fed to cattle, the levels were from 200 to 600 mg. per head per day. In the following years there have been reported many feed-lot trials with various levels and there now exist considerable data on chlortetracycline and oxytetracycline to show that at 75 to 150 mg. per head per day these antibiotics promote substantial increases in growth and feed efficiency without adverse side effects.

Experiments with chlortetracycline in feeding of beef animals up to about the middle of 1954 have been reviewed by Elliott[15] and there have been several reports since then.

Within recent months, a substantial amount of data on oxytetracycline for beef cattle has become available. These include trials conducted by agricultural colleges, by Chas. Pfizer & Co., at its Agricultural Research Center, near Terre Haute, Ind., and field trials.

The highlights of these studies are given in table XI.

In these extensive feeding trials there has been noticed a slight initial decrease in feed intake when steers first are placed on feed containing the antibiotic. This perhaps reflects the transient alteration in rumen cellulolytic capacity that was discussed in the section on rumen function. However, feed intake rapidly returns to normal as reflected by the accelerated weight gains. Absolute weight increment is comparable on low- and high-roughage programs of feeding. Oxytetracycline is effective over the entire growing fattening period. In several studies it was observed that among animals receiving oxytetracycline there was better hair coat and less incidence of bloat and of liver damage.

Growth of Sheep. In general, growth studies of antibiotics with sheep have yielded results of wider variability than those from comparable cattle-feeding experiments.

A review of growth studies with chlortetracycline for lambs has been presented by Elliott and Maddock.[16] Recent work with oxytetracycline is summarized in table XII.

A collateral beneficial effect of antibiotic feeding to lambs noted in several studies has been reduction in enterotoxemia (overeating disease). Also, in the case of oxytetracycline, it has been found by Sacchi et al[17] that this antibiotic in the feed permitted continued consumption of feed and gain in weight of lambs suffering from an outbreak of contagious ecthyma (sore mouth disease).

MODE OF ACTION OF ANTIBIOTICS IN PROMOTING GROWTH

Many theories have been advanced to explain the growth-promoting action of antibiotics for livestock and poultry. There is much to support the concept that a more nearly "normal" growth potential is realized because the antibiotics check unknown subclinical infections. This theory is predicated on the observation that the greatest weight gains are usually in groups of animals suffering from scours and other evident disease. Conversely, growth stimulatory effects have been found to be minimal, or even nonexistent in animals kept under "disease-free" conditions.

In this connection there may be noted the work of Whitehair and Thompson[18] with young pigs and of Coates et al[19] with poultry. These and other workers also have reported that animals or birds under "disease-free" conditions, or those under

TABLE XII

Oxytetracycline in Rations for Lambs, 10 Gm. to 25 Gm. per Ton of Feed

	Controls	Oxytetracycline	Per cent improv.
Summary of Growth and Feed Efficiency			
Growth			
No. trials	5	5	—
Av. daily gain, lb.	0.415	0.469	11
Feed Efficiency			
No. trials	5	5	—
Av. lb. feed/lb. gain	9.45	8.58	9.4
Summary of Carcass Quality Data			
No. trials	4	4	
Av. score	9.10	9.16	
No. trials	3	3	
Av. dressing %	51.03	51.4	
Summary of Economic Data			
No. trials	5	5	—
Av. cost/lb. gain, $	0.189	0.178	5.8
Av. incr. ret./head	—	—	$0.432

usual conditions but fed antibiotics, appear to have a more healthy intestinal wall than animals that have not received antibiotics but are kept in usual environments. Pathogenic organisms, whether bacterial, protozoal, or viral, can be presumed to interfere with maximum efficiency of the complex mechanisms, both chemical and physical, that operate in the conversion of food to living tissue.

Considerable emphasis has been given also to the finding that the antibiotics have a sparing effect on some nutrients—especially protein and certain vitamins. It is not established whether this is a true nutrient sparing action or an indirect effect, in that the antibiotics in the feed are conducive to a more healthy digestive tract, which thereby permits more efficient digestion and absorption of nutrients.

Another avenue through which antibiotics may contribute to improvement of nutrition is through stimulation of appetite. A good appetite is one of the important accompaniments of health, and it is known that antibiotics at low levels in the ration increase feed consumption in chicks, pigs, and calves.

SUMMARY

Antibiotics are of established value for speeding growth of many species of animals and birds. The accelerated weight gains usually are made with less consumption of feed per pound of gain.

The mode of action is not known, but there is much to favor the theory that the improvements in animal production with antibiotic feeding are due to control of known or unknown pathogens, thus improving general health and providing opportunity for a more nearly maximal growth potential to be realized. In fact, under practical conditions, it is often the lowering of mortality within a herd and improvement of general condition that attracts the attention of the livestock producer as much as weight gains and economy of feed conversion.

The subject of the role of antibiotics in nutrition is complex and experience to date indicates that, in conducting feeding trials with antibiotics or interpreting data of such trials, one must give careful consideration to environment, other dietary factors, and the general health of animals.

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The Antibiotic Regulations for Medicated Feeds

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Drugs that contain one or more of the antibiotics penicillin, streptomycin, dihydrostreptomycin, chlortetracycline,* tetracycline, bacitracin, and chloramphenicol† are subject to the certification requirements of sections 502(1) and 507 of the Federal Food, Drug, and Cosmetic Act. As such, each batch must be certified by the Food and Drug Administration prior to its shipment in commerce, unless the drug has been exempted from such requirements by regulations issued by the Secretary and published in the *Federal Register*. Before regulations can be issued to provide either for the certification or exemption from certification of an antibiotic drug, data must be submitted to the Food and Drug Administration adequate to prove that the drug is safe and efficacious for use under the conditions proposed in its labeling.

In the latter part of 1950 and the early part of 1951, the Food and Drug Administration was shown the results of a series of experiments that proved conclusively that antibiotics incorporated in feeds, under usual field conditions, increased the rate of growth of certain species of animals and were safe for use for such animals. The question was then raised as to whether an antibiotic feed intended for use solely to promote rate of growth was in fact "a drug." While the mode of action of antibiotics as a growth stimulant was not known, the indications were, at that time, that the growth effect was due to their antibacterial or drug action. There is evidence now to show that the growth response results from a double-barreled drug effect: the antibiotics apparently inhibit the growth of pathogenic, nonpathogenic, and toxin-forming microorganisms that are injurious to the animal and, by their modification of the intestinal flora, the organisms may promote either the synthesis or sparing, or both, of critical nutrients needed by the animal. Having reached the conclusion that an antibiotic feed is a drug as well as a food, such feeds were therefore subject to the antibiotic provisions of the act. Thus, before they could be marketed lawfully, it was necessary to amend the antibiotic regulations to provide either for their certification or to exempt them from such requirements. It was agreed that predistribution testing of each batch and certification of such preparations was not necessary to insure their safety and efficacy of use if they were intended solely to promote rate of growth. The antibiotic regulations were so amended April 28, 1951, to include what is now section 146.26, which exempts such feeds from certification.

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* The trade name of American Cyanamid Co. for chlortetracycline is Aureomycin.
† The trade name of Parke, Davis & Co. for chloramphenicol is Chloromycetin.

After it was shown that antibiotic feeds are effective in promoting rate of growth, we next received clinical data adequate to prove that, at certain concentrations, they were also effective in the prevention or treatment of certain infections in poultry, swine, and calves. Such preparations were exempted from certification only if they contained the prescribed amounts of specific antibiotics and if they were represented for use only in the prevention and treatment of the diseases specified in the exemption. These conditions for exemption were subsequently extended to include antibiotic feeds containing antibiotics and certain active drug components that were incorporated for their specific disease control effects.

Until the regulations were amended to provide for the use of the coccidiostat, nicarbazin, as an ingredient of an antibiotic-containing feed, it was not necessary for the feed manufacturer to engage in any preliminary formalities with the Division of Antibiotics because the active nonantibiotic drug components enumerated by the antibiotic regulations for use in antibiotic feeds had lost their new drug status. This was not the case with nicarbazin. Since it was and still is in a new drug status, it and feeds containing it are subject to the new drug provisions of section 505 of the Act. However, under the provisions of the Act, if a certifiable antibiotic is added to nicarbazin or to any other new drug, the new drug is no longer controlled by section 505 but by the certification provisions of section 507. Therefore, if the same type of regulatory control were to be maintained for an antibiotic-nicarbazin mix as it was for a nicarbazin mix without antibiotics, it would be necessary for the feed manufacturer to show the Commissioner that his preparation is safe and efficacious before it is shipped initially in commerce. Such proof would consist of the results of the clinical investigations made on the preparation, a description of the methods and processes used in its manufacture and control, and specimens of all labeling to be used for it. The regulations that were issued to exempt antibiotic-nicarbazin mixes from certification include these provisions. They differ from those previously issued in that they exempt from certification antibiotic-nicarbazin mixes of individual manufacturers rather than providing for carte blanche exemption of these drugs.

Since nicarbazin, the regulations have been amended in the same manner to exempt antibiotic feed mixes that contain 2-acetylamino-5-nitrothiazole, arsenobenzene, dienestrol diacetate, 2,4-diamine-5-(*p*-chlorophenyl)-6-ethylpyrimidine, or sulfaquinoxaline.

No single section of the antibiotic regulations has been more active than section 146.26. Since it was first issued until January 1956, it has been amended 38 times to include new preparations or new uses for old ones. This is not too surprising, however, when we consider the enormous quantities of poultry and livestock feed used in this country and the percentage of these feeds that contain one or more antibiotics. The Association of the American Feed Manufacturers estimates that, in the year 1954, the country's animal feed requirements were about 115,000,000 tons, of which 35,000,000 tons were supplied by the feed manufacturers and the remainder grown and mixed by the farmer. In this same year, according to the United States Tariff Commission, 562,000 pounds of antibiotics, valued at $26,000,000, were sold for use in animal feed. If the average antibiotic feed contained 10 Gm. of the antibiotic/ton, this quantity of antibiotics was sufficient to prepare almost 26,000,000 tons of antibiotic feeds. Therefore, about 22 per cent of the feeds used in 1954 contained antibiotics. This percentage was considerably

higher for feeds supplied by the feed manufacturers. While the figures for 1955 are not yet available, from our experience with these preparations, they should show a substantial increase.

In conclusion, feeds containing certifiable antibiotics, regardless of their intended use, are subject to the certification provisions of the Act. Therefore, before marketing such feeds, the manufacturer should assure himself that the antibiotic feeds he makes are exempt under section 146.26 of the antibiotic regulations and that they are strictly compounded to comply with the exemption. We will be glad to assist any manufacturer in any way we can in bringing his preparations in compliance with the law.

Poultry Feed as a Method of Anthelmintic Medication

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During the past two decades, there has been an increasing tendency toward larger size flocks of poultry. Today it is common to find flocks of 10,000 broiler chickens, 1000 laying hens plus replacement stock, and 5000 turkeys. As might be expected with this increase in flock size, there has been an increase in helminth infection; particularly with the large roundworm, *Ascaridia galli*. This is largely attributable to an increase in density of population in a confined area.

Since the individual bird is of low economic value and the labor of administering an individual treatment to a large flock of birds is considerable, it has been economically feasible to develop a method of mass treatment. The two possible vehicles for such treatments are the feed and the drinking water. It is only during the last year that satisfactory treatments have become available through the latter vehicle, while the use of feed as a vehicle has been increasing during the past 10 to 15 years. Today, feed is a very important means of administering anthelmintic medications.

The beginning of feed medication occurred with the report of Hermes and Beach[1] who, in 1916, stated that steep water from tobacco stems was effective in removing the large roundworm from poultry when mixed with feed to make a wet mash. The disadvantage of this and tobacco dust, which was subsequently used, is the impossibility of controlling the dosage of nicotine because of the variation of nicotine content in the dried plant material. Too high a dosage of nicotine would be quite toxic to the chicken and too low a dosage not effective. Today, nicotine-containing wormers consist of nicotine sulfate adsorbed on some type of inexpensive adsorbent.

Repeated testing has shown that the amount of nicotine alkaloid required per bird to cause a 90 per cent or better removal of the large roundworm, *A. galli*, from the chicken, regardless of age, is 50 mg. It is difficult for some to realize that it is not necessary to increase the dosage for larger birds. In a sense, one is treating the worm and not the bird, for successful treatment does not require adsorption of the drug into the host tissue but only the release of the nicotine in the environment of the worm so that its motility is reduced. Kerr and Cavett[2] have shown this to be the effect of nicotine on this species of helminth. With reduced motility, the worm cannot maintain its position in the intestine and is swept out by normal bowel action.

The importance of the adsorbing agent for the nicotine is shown in table I. Ten different sources of Fuller's earth, seven sources of bentonite, and two resins

TABLE I

Effect of Adsorbing Agent on the Anthelmintic Activity of Nicotine

Nicotine consumed, mg.	Adsorbing agent	No. birds	*Ascaridia galli*		
			Total	Removed	% removal
49	Fuller's earth 1	5	34	30	82
45	Fuller's earth 2	8	113	77	68
50	Fuller's earth 3	10	195	154	79
55	Fuller's earth 4	10	78	39	50
55	Fuller's earth 5	10	219	164	75
55	Fuller's earth 6	9	294	251	85
50	Fuller's earth 7	9	271	167	62
54	Fuller's earth 8	8	209	157	75
55	Fuller's earth 9	7	246	146	59
45	Fuller's earth 10	9	200	151	76
51	Bentonite 1	8	226	98	43
47	Bentonite 2	6	39	24	62
58	Bentonite 3	8	108	79	73
52	Bentonite 4	8	77	56	73
46	Bentonite 5	7	114	47	41
46	Bentonite 6	7	315	307	97
51	Bentonite 7	8	180	175	97
45	Resin 1	8	66	66	100
52	Resin 1	8	122	106	87
50	Resin 2	9	275	114	41

were used. The dosage of nicotine alkaloid varied from 45 to 58 mg. per bird. The activity obtained for the Fuller's earth-nicotine mixtures varied from 50 to 85 per cent; and for the bentonite-nicotine mixtures, from 41 to 97 per cent; and that for the resin-nicotine mixtures, from 41 to 100 per cent. It is obvious that in the preparation of a nicotine-containing product care should be used in the selection of the adsorbent if a quality product is to be provided.

A disadvantage of nicotine as an anthelmintic is that it is primarily active only against the maturing and mature worms. Table II gives the results of three tests illustrating this point. It will be noted in the table that it is not until the worms are 3 weeks old that any significant reduction in infection was found, and the activity was not so great as when the worms were 4 weeks old.

The question is frequently raised as to the length of time the medicated feed should be administered, that is, should the medicated feed be administered for one day or more than one day, assuming the required per bird dosage of 50 mg. of nicotine is to be consumed. This problem is related to the daily feed consumption of the birds and to the palatability of the medicated feed. It is our experience that for young birds the limiting factor is the acceptance of nicotine-medicated feed. Feed containing 0.1 to 0.12 per cent nicotine is reasonably well accepted by 5 week old broilers, but at higher levels the feed consumption drops off markedly. Even at this level the feed consumption may be reduced as much as 25 per cent below normal.

With regard to laying birds, our recommendations have been a lower level of nicotine in the medicated feed. A nicotine level of 0.025 to 0.05 per cent is gener-

ally recommended because the acceptance of the medicated feed is better and there is thus less chance of causing a reduction in the rate of egg production due to a reduced feed consumption. Adult chickens will consume the proper dosage of nicotine in one to two days when it is incorporated in the feed at the levels indicated.

Lower levels of nicotine can be used in the feed preparation. The efficiency of action is good provided the medicated feed is administered over a sufficient period of time to provide an effective dosage of nicotine. We believe the medication should be completed within a maximum of four to five days; however, most feed manufacturers prefer to use the higher levels administered over a shorter period of time.

The fowl cecal worm or pinworm is the second most commonly found worm. The specific drug for its removal is phenothiazine, the activity of which was first reported by Harwood[3] in 1938. Like nicotine, a standard dosage has been established, which is stated in the *National Formulary*[4] as 500 mg. per bird. This dosage provides an efficacy in the vicinity of 95 per cent. The amount of phenothiazine usually provided in feed is 10 times that of nicotine, varying from 0.25 to 0.5 per cent.

The amount of phenothiazine that can be administered in feed is regulated, as is the case with nicotine, by the acceptance of the medicated feed on the part of the birds. Again, it makes little difference in degree of activity if the proper dosage of phenothiazine is administered in one or several days.

It should be pointed out that phenothiazine may cause a red to purplish discoloration of the droppings. This is sometimes alarming to the poultryman because he thinks the discoloration is due to hemorrhage. The discoloration is due to the excreted form of the drug.

In recent years two compounds for which claims of the removal of tapeworms are made, have been offered for mixing in the feed. The activity of these compounds against three of the more important species of tapeworms are presented in

TABLE II

Efficacy of Nicotine Wormers in Removing Ascaridia galli *of Different Worm Ages*

		Test number					
		1		2		3	
No. birds /test group	Age of worms, wk.	Nicotine consumed, mg.	Av. no. worms found	Nicotine consumed, mg.	Av. no. worms found	Nicotine consumed, mg.	Av. no. worms found
10	4	None	20.3	—	—	None	18.6
10	3	—	—	None	72.1	—	—
10	1	76	27.2	64	37.4	48	27
10	2	69	16.3	62	62.9	47	15.3
10	3	59	12.1	62	28.4	80	5.7
10	3	—	—	58	8.2	—	—
10	4	52	2.2	—	—	56	1.1
10	4	52	6.3	—	—	—	—

tables III, IV, and V. These data are taken from publications by Kerr,[5] Kerr and Green,[6] and Edgar.[7] It will be noted in these tables that dibutyltin dilaurate was the only compound with a consistently high degree of activity against all three species of tapeworms, *Raillietina cesticillus, Choanotaenia infundibulum,* and *Davainea proglottina.* The first of these is considered to be the most commonly found species of tapeworm. Both the compounds mentioned have a high degree of safety for chickens and turkeys and are completely compatible with nicotine and phenothiazine. All of these compounds can be mixed with feed to be passed through pellet mills. This process does not cause a loss of active ingredient.

Repeated testing in the laboratory and in well-managed field flocks has not resulted in a reduction in egg production when the combinations of these drugs are administered at the proper dosage. Birds in extremely high production, more than 75 per cent, may show a temporary loss in egg production, but it is difficult to understand why such birds should be treated. It has been our experience that a greater loss in egg production has occurred through an abrupt change in type of feed or feeding practice than can be attributed to anthelmintic medication given in the usual feed and by the usual feeding practice.

Within the last year, the piperazine compounds have been marketed as anthelmintics. These have been used principally for water medication but can be used for feed medication. The extent of their use in poultry feeds in this country is minor at the present time. Piperazine possesses a remarkable activity against the large roundworms and, in our opinion, is the safest anthelmintic known today.

TABLE III

Activity of Several Compounds in Removing the Tapeworm Raillietina cesticillus *from Chickens*

	Dosage		No. infected birds	Necropsy findings		
	Mg./Kg.	Mg./bird		Total no. tapeworms	Av. no. tapeworms	Indicated efficacy, %
2,2'-Dihydroxy-5,5'-dichlorodiphenylmethane						
	50	—	8*	193	24.1	5
	100	—	8*	159	19.9	22
	150	—	8*	148	18.5	27
	200	—	8*	198	24.7	3
Unmedicated controls			8*	203	25.4	—
	—	725	2*	17	8.5	42
Unmedicated controls			6*	88	14.67	—
	—	375	4†	147	36.7	54
Unmedicated controls			5†	398	79.6	—
Dibutyltin dilaurate						
	—	112	5‡	0	0	100
Unmedicated controls			5‡	57	11.4	—
	—	87	8‡	14	1.75	98
Unmedicated controls			8‡	640	80	—
	—	150	11†	0	0	100
Unmedicated controls			11†	46	4.2	—

* Kerr and Green.[6]
† Edgar.[7]
‡ Kerr.[5]

TABLE IV

Activity of Several Compounds in Removing the Tapeworm Choanotaenia infundibulum *from Chickens**

	Dosage		No. infected birds	Necropsy findings		
	Mg./Kg.	Mg./bird		Total no. tapeworms	Av. no. tapeworms	Indicated efficacy, %
2,2'-Dihydroxy-5,5'-dichlorodiphenylmethane						
	—	722	4	4	1	94
Unmedicated controls			4	66	16.5	—
	—	750	3	0	0	100
Unmedicated controls			5	29	5.8	—
Dibutyltin dilaurate						
	—	125	3	0	0	100
Unmedicated controls			5	29	5.8	—
	—	82.4	4	0	0	100
Unmedicated controls			4	22	5.5	—

* Data for this table taken from Edgar.[7]

Some claims have been made for its activity against the cecal worms; however, in our testing we have failed to find a consistent effect. Using a dosage effective for the large roundworm, the maximum removal obtained by us has been in the vicinity of 40 per cent, and in some cases no removal has occurred.

As a result of testing several salts of piperazine, we have reached the conclusion that the activity of the compound used depends entirely on the amount of piperazine contained in the salt. For this reason, it is our opinion that the labeling of piperazine-containing products should be standardized to state the amount or percentage of piperazine, just as nicotine-containing products state the amount or percentage of nicotine.

Mention should be made of the purpose of administering a feed containing an anthelmintic. The purpose of much medication is certainly the removal of existing infection. Frequently, the existing infection is not great enough to cause serious damage to the birds, and the question, "Why treat?" may be raised.

TABLE V

Activity of Several Compounds in Removing the Tapeworm Davainea proglottina *from Chickens**

	Dosage		No. infected birds	Necropsy findings		
	Mg./Kg.	Mg./bird		Total no. tapeworms	Av. no. tapeworms	Indicated efficacy, %
2,2'-Dihydroxy-5,5'-dichlorodiphenylmethane						
	—	750	4	1488	496.0	0
Unmedicated controls			4	1903	475.7	—
Dibutyltin dilaurate						
	—	125	4	209	69.6	85
Unmedicated controls			4	1903	475.7	—
	—	92	5	9	1.8	99
Unmedicated controls			5	1032	206.4	—

* Data for this table taken from Edgar.[7]

TABLE VI

*Results of Administering Nicotine at Low Levels in the Feed Continuously for 10 Weeks to Prevent Infection with the Large Roundworm**

	A	B	C	D
Nicotine, % in feed	0.00275	0.0055	0.011	none
No. birds				
At start	24	25	24	24
At completion	22	23	22	19
Av. weight gain, Gm.	949	838	875	843
Total feed consumption, Gm.	68710	64760	63040	61610
Total worms	741	1209	989	1101
Av. no. per bird	33.7	52.6	44.9	57.9
Per cent birds infected	91	96	95	95

* Medication started when birds were 2 days old. Each bird was exposed to an infection of 200 embryonated ova six days a week for six weeks, starting when the bird was 8 days old.

There is considerable evidence that the large roundworms and cecal worms do more damage during the tissue-invading stage of their life cycles. A treatment to reduce a minor existing infection serves the purpose of reducing the contamination of the environment with worm eggs. Thus it reduces potential reinfection and the damage caused by the very young worms. More heavily infected birds are directly benefited.

An additional concept in worm control is the prevention of infections through the continuous administration of a drug through the feed. Temperton and Dudley,[8] Wehr and Olivier,[9] and Griffiths[10] have all shown that the continuous feeding of phenothiazine at low levels and therapeutic levels (1 and 2 per cent) were ineffective in preventing the establishment of either the large roundworm or the cecal worm in chickens and turkeys. Likewise, low levels of nicotine are ineffective in preventing infection with the large roundworms, as is shown in the data presented in table VI. The highest level of nicotine used in this test (Group C) is one tenth of a therapeutic dose.

It is interesting to note that piperazine, when administered in the feed continuously at levels of a tenth or less of the therapeutic dose, is also ineffective in preventing infection with the large roundworm. The data in table VII illustrate

TABLE VII

*Activity of Piperazine in Preventing Infection with the Large Roundworm When Administered at a Low Level in the Feed.**

	A	B	C	D	E
Piperazine, % in feed	0.0088	0.0058	0.0029	0.0012	none
No. birds	10	10	10	10	20
Total feed consumption, Gm.	6770	6570	6730	7490	13140
Mg. piperazine consumed/bird	60	38	20	9	—
Av. weight gain, Gm.	762	267	314	252	286
Total worms	166	173	210	333	424
Av. no. worms	16.7	17.3	21.0	33.3	21.2

* Single infection of 450 embryonated ova per bird. Medication was continued for three weeks.

TABLE VIII

*Worm Infections, Chickens: Effect of Polystat 0.2 Per Cent in Feed Continuously, Providing 200 ppm Dibutyltin Dilaurate, in Preventing the Establishment of the Infections**

Test no.	Group medication	No. infected birds/no. at completion	Worm burden: Total per group	Worm burden: Av. bird in group at completion	Worm burden: Range infected birds	Indicated prevention, per cent
Ascaridia galli						
63–4–54†	Medicated	1/25	1	0.04	1	99
	None	17/24	117	4.8	1–59	—
63–6–54‡	Medicated	0/25	0	0	0	100
	None	11/23	65	2.8	1–15	—
63–8–54‡	Medicated	0/24	0	0	0	100
	Medicated	0/24	0	0	0	100
	None	14/24	60	2.5	1–15	—
63–10–54‡	Medicated	4/25	31	1.2	2–18	92
	None	21/25	395	15.8	1–103	—
63–12–54§	Medicated	2/25	3	0.12	1–2	99
	None	24/25	489	20.3	5–38	—
Raillietina cesticillus						
63–4–54‖	Medicated	0/25	0	0	0	100
	None	24/24	503	20.9	2–46	—
63–6–54‖	Medicated	1/25	1	0.04	1	99
	None	23/25	538	23.4	5–75	—
63–10–54‖	Medicated	0/25	0	0	0	100
	None	25/25	759	30.4	10–65	—
63–12–54¶	Medicated	0/25	0	0	0	100
	None	24/24	865	36.04	8–78	—

* There were 25 birds/group. Medication was started at 3 days of age and continued for 10 weeks. All surviving birds were killed and examined for worms when 10 weeks old.

† Dosage was 200 embryonated eggs/bird on feed, given six times at weekly intervals, starting at 10 days of age.

‡ Dosage was 200 embryonated eggs/bird on feed, given 12 times, twice weekly, starting at 10 days of age.

§ Dosage was 600 embryonated eggs/bird, given 12 times, twice weekly, starting at 10 days of age.

‖ At 5 weeks of age, each bird received a single infection of 50 cysticercoids in *Tribolium confusum*.

¶ Bird received infection of 50 cysticercoids in *Tribolium confusum* at 5 and 7 weeks of age.

this point, for the birds on the highest level of medication given harbored an average of 16.7 worms while the nonmedicated controls harbored an average of 21.2 worms.

During the past year, a product was introduced that claims to be an aid in the prevention of infection with the large roundworms and tapeworms. This product, Polystat, when used at the level of 0.2 per cent in the feed, provides 200 parts per million dibutyltin dilaurate, the ingredient active in worm prevention. Table VIII summarizes data on this point. The indicated prevention of the large roundworm in the five tests varied from 92 to 100 per cent, and the tapeworm infections were almost completely prevented. These data show that prevention of worm infection through medicated feeds is possible. It is reasonable to predict that other compounds having the same or a more pronounced preventive effect will be found.

In spite of the recent development of the piperazines and the use of water as a vehicle for anthelmintic medication, feed will continue to be used for this purpose. Medication through the feed has the advantage of more accurate control of the medication ingredients because feed manufacturers are accustomed to producing a product that contains small amounts of important ingredients properly distributed through their product. Also, under the present conditions of raising poultry, the amount of medicated feed to be given to a certain number of birds is easier to control than is the amount of medicated water.

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Anthelmintics in Animal Feeds

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Feeds are an essential factor in livestock production. They represent man's improvements over the forage on which the forebears of his domesticated animals subsisted and are designed to increase the efficiency of the physiologic processes from which livestock products are obtained. Their efficiency is dependent to a considerable extent upon breeding, management, disease, and parasitism.

Even our cleverly bred and carefully managed animals are far from ideal as converters of feed. We are forced to cope with a conglomeration of physiologic processes and biologic interactions related only indirectly to the desired end products. For example, we must feed to build inedible skeletons upon which to hang marketable red meat. We must reckon with helpful and harmful flora and fauna of the digestive tract.

Thus, modern feeding must take into account not only the balance of nutrients suitable for the ideal situation but anything and everything that in one way or another enhances feed efficiency under practical conditions. A growth promoter need not be an essential metabolite. It may, for example, speed up or slow down advantageously a critical metabolic process. It might encourage a cooperative flora or discourage an antagonistic fauna. Feeds are essentially combinations of fuel and raw materials, which, in our present ignorance, must be processed in the digestive tracts of our sheep, cattle, and swine in order to obtain the livestock products we are after. We have passed the point at which only the so-called natural feed materials rate consideration as feed ingredients. A modern feed may owe its efficiency to the incorporated counterparts of the catalysts, lubricants, and antiknock agents of modern fuels. Within the limits set by economics, we seek the magic balance of ingredients that will give the best results.

This is a real challenge, this compounding of a prescription for more efficient production or for "superhealth." We are challenged, first, to make full use of the materials in hand today and, second, to discover new ones. Let us not be discouraged in the latter effort by the thought that the evolutionary string may be nearly run out in our present-day domesticated animals. One can imagine that the very hormones arising from time to time in their prehistoric pasts to boost them on their evolutionary ways would no doubt have been viewed with alarm by a paleontologic pathologist. Though it is true that ruminants are ruminants, for example, it is also a fact that ruminants have not been exposed to a vast array of chemicals synthesized by man in recent times. Among the latter, when we get

around to trying them out, will no doubt be discovered some that modify ruminant biology to the advantage of production. While pioneering research is seeking out new agents for increasing feed efficiency, we can apply some of the old ones. The use of anthelmintics in feeds is a good example of the latter.

HELMINTHS AS A PRODUCTION HAZARD

Parasitized animals are poor risks if the objective is maximum efficiency. This is obvious when the animals are suffering from severe parasitism. No feed dealer wants to risk the reputation of his feed on a scouring and worm-ridden herd. The same is true, though the hazard is less obvious, with subclinical parasitism.

Significant features of helminth parasitism in relation to livestock production have been reviewed elsewhere.[1] These cannot be detailed here, but the following generalizations are pertinent to the present discussion. The degree of damage inflicted by helminths is basically proportional to the numbers attacking the host. Because worm parasites exploit their hosts to grow to maturity rather than to multiply their number, it follows that severity of parasitic infection is directly related to the extent of exposure of the host to the nonparasitic, but infective, stages of the life cycle of the worm. Parasitism is essentially a disease of the herd as a whole; it characteristically builds up or incubates in the herd slowly. During this process, the immunity that is stimulated in individuals is unreliable for protecting the herd, and the low-grade infections are often an economic burden in themselves. Control by prevention of parasite population build-up is suggested by the nature and behavior of worm parasitism in livestock.

Under practical conditions, the significance of low-grade infection in healthy looking animals can be demonstrated only by comparing their production with that of comparable animals harboring fewer parasites. When the treated half of a split herd outperforms the untreated half, the anthelmintic has been discovered, so to speak, as a missing factor in production. This is the same technique employed to demonstrate the advantage of an antibiotic or a hormone. When increased growth and/or efficiency results, it is only academic whether one speaks of deficiencies or of additives.

PARASITE CONTROL AS AN AID TO PRODUCTION

Many of the experiments and field tests that demonstrated the economic losses due to helminths have at the same time demonstrated the economic advantage resulting from parasite control. The simple device mentioned, of comparing weight gains in treated and untreated portions of naturally infected herds under routine management, has been helpful in pointing up the significance of even relatively mild parasitism. When the extra gains result in profit over and above the cost of treatment, the parasitism involved may be considered a drag on production, and parasite control makes economic sense.

In the case of mixed infections with gastrointestinal worms in cattle, for example, treated groups gained from 0.06 to 0.40 lb./head/day more than comparable untreated groups, representing extra profits of 2 to 12 dollars/head for the relatively short periods involved. These herds were not heavily parasitized; the major effect of their parasite burdens was simply a depression of weight gains.

The modern emphasis on improved efficiency in livestock production puts a premium on parasite control. The feed manufacturer is particularly involved because the full potential of his feed is not realized when it competes with internal parasites. Helminth infections no longer have to be spectacularly destructive to bother the alert producer who is looking for maximum performance from the feed he buys and the management he practices. Economic considerations have put feeding and parasite control on the same production team. In some cases, the combination works most effectively when the anthelmintic is incorporated in the feed.

FEEDS AS VEHICLES FOR ANTHELMINTICS

Feeds have been used for many years as carriers of therapeutic doses of anthelmintics. Pigs are commonly wormed with sodium fluoride mixed with their feed. Commercial mixtures of feed and one or more anthelmintics are known to the trade as "wormers." Herd treatment by way of the feed, although lacking the uniformity and sureness of individual dosing, has proved to be practical. Its low cost and ease of application make it the method of choice in many instances. The feeding programs recommended by feed manufacturers today often include treatment for worms with their own products.

Feeds are also a natural vehicle for carrying into the body any substance we wish to supply on a day-to-day basis, be it an essential vitamin, a stimulating hormone, or a prophylactic drug. Thus, there has developed the use of feeds containing low levels of phenothiazine for preventing the build-up of parasitic infection in the herd.

It goes without saying that the public health is of first concern in the use of any drug and that whoever employs a drug in animal production will be guided by appropriate knowledge of its acute and chronic toxicity, excretion and storage, and side effects.

PHENOTHIAZINE AS A PREVENTIVE ANTHELMINTIC

Phenothiazine, being the only anthelmintic widely employed as a helminth prophylactic today, can serve as an example for illustrating some of the technical considerations that must be taken into account in the manufacture and use of medicated feeds designed for parasite prevention. The anthelmintic properties of phenothiazine have been reviewed by Harwood,[5] its chemistry by Massie,[6] and its role as a low-level preventive by the writer.[1]

In the first place it must be recognized that phenothiazine is not a prophylactic in the strict sense of the term. It is not particularly effective against the infective larvae that first enter the host.

Phenothiazine owes its success as a parasite control agent primarily to its activity against the postadult stages of a large variety of gastrointestinal worms of sheep, horses, and cattle. To this long list, it may be possible to add in the near future certain species parasitic in swine and dogs. Low levels of phenothiazine in the diets of the host species mentioned either reduce the egg production of the worms or render the worm eggs incapable of developing into infective larvae, depending upon the worm species involved. The result of these suppressive activities is to

cut down the parasite population and hence to reduce exposure to reinfection. This is prophylaxis after the fact of first infection, which, although not so desirable as prophylaxis in the strict sense, has nevertheless successfully been adapted to practical regimens for controlling helminths of livestock. Furthermore, there is evidence that continuous medication with small daily doses reduces the original worm burdens, representing what one may term a prolonged therapeutic action.

The broad spectrum of activity against worm eggs and larvae possessed by phenothiazine augurs well for its continued usefulness. Its general effectiveness against these postadult stages of nearly all of the economically important roundworms of ruminants, for example, makes differential diagnosis less important for practical control regimens than for prescribing specific therapy. As a matter of fact, it has been observed that continuous free-choice phenothiazine in a closed flock of sheep was accompanied by the disappearance of lungworms, a species in which the adults are not removed by the drug. The parasites are often thus defeated by attrition.

The relatively low toxicity of phenothiazine for vertebrates and its rapid excretion are also in its favor as a preventive drug to be fed at low levels. It has been given to experimental flocks of sheep for 10 and 8 year periods at average daily consumption rates of slightly more than 1 Gm. and slightly less than 0.5 Gm./head, respectively.[7, 11] Horses have received 2 Gm. daily for four years.[9] Cattle have been fed 2 Gm./day for a year[3] and four times this amount for two months.[2] No evidence of gross or microscopic pathologic changes, including any in the blood, has been reported in the animals on these levels of daily phenothiazine consumption. Dogs[4, 10] and swine[8] have likewise been shown to tolerate low levels of the drug given continuously for extended periods.

CONCLUSION

Whenever livestock production requires treatment or prevention of helminth infection by means of drugs in feed, the feed manufacturer approaches the field of parasitic disease staked out by the professions of parasitology, animal pathology, and veterinary medicine. Economic considerations will determine the who and the how of disease control in livestock production. Just as the veterinarian must call on the knowledge of the nutritionist to speed his convalescing patients back to health, so must the feed manufacturer utilize applicable disease prevention techniques to make feeding efficient. There is no way forward except to pool our knowledge and apply it where and how it makes the most sense in livestock production.

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New Drug Status of Medicated Feeds

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The ever-increasing demand for an abundant and more economic food supply has stimulated the employment of means for producing meat quicker with less natural feed by utilizing hormones, antibiotics, and other drugs. This has created new problems in protecting the food supply for both man and animals from adulteration with toxic or deleterious ingredients.

One of the most practical ways to administer drugs to a large group of animals or fowls is to mix them into the feed. Many manufacturers of livestock and poultry feeds have placed on the market feed mixtures containing drugs for physiologic or therapeutic purposes. Such mixtures come under the definition of a drug and must comply with the drug provisions of the Federal Food, Drug, and Cosmetic Act. As such, they are subject to both the safety and labeling requirements of the law. In consequence, feed manufacturers have become drug manufacturers with drug problems that also affect their suppliers and control officials.

In addition to articles recognized in the official compendia, the Act contains two statutory or legal definitions for drugs: (1) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and (2) articles (other than food) intended to affect the structure or any function of the body of man or other animals. Thus, under legal definition, we have two main categories of drugs: (1) those used for diagnosis, prophylaxis, or therapy of disease; and (2) those used for a physiologic effect.

Because of the tremendous amount of research done within the past few years, many new therapeutic and physiologic agents have been developed, most of which fall within the definition of the term "new drug" in the Act. Newer and better drugs are being discovered and will continue to be discovered. The use of these drugs in feeds creates new problems of safety. The average consumer does not have the qualifications and the facilities to investigate the safety of drugs or to evaluate the safety of products from animals fed medicated feeds. They must rely on the integrity of drug and chemical manufacturers and the concern and watchfulness of Government agencies.

The term "new drug" is defined by the Act as any drug that is not generally recognized, among experts qualified to evaluate the safety of drugs, as safe for use under the conditions prescribed, recommended, or suggested in its labeling. The article remains a new drug until it has been used for a material time and to a material extent, apart from its use in investigations establishing its safety.

Most manufacturers realize that a new drug cannot be marketed legally in interstate channels until a new drug application with respect to it becomes effective as provided by section 505 of the Act. Section 505 (i) provides for exemptions for drugs intended solely for investigational use. Any manufacturer who is interested in obtaining satisfactory evidence for the submission of a new drug application should carefully consider this exemption. In this connection research workers engaged in such studies should bear in mind that they may unwittingly be producing adulterated food unless they determine that the edible products from experimental animals are free of drug residues.

It is important for all drug manufacturers and distributors to give careful consideration to the definition of a "new drug." It is the responsibility of the manufacturer or distributor to decide whether the particular drug or combination of drugs in which he may be interested comes under this definition. If the manufacturer is unable to determine for himself whether a preparation is a new drug, we will be glad to give an opinion if we are furnished the quantitative composition of the preparation and specimens of the proposed label or labeling.

We recognize that substances that possess prophylactic and therapeutic merit for the prevention and treatment of animal and poultry disease are necessary in the production of meat used for food. Although some of these drugs are highly toxic to humans, new drug applications for them are made effective when the applicant submits convincing evidence that when used as directed the amount of the drug left in edible products is too small to have an adverse effect on human health.

Our position in relation to drugs intended for purposes other than for the prevention or treatment of disease is significantly different. As a matter of policy the Department of Health, Education, and Welfare takes the view that drugs intended to affect physiologic functions, for example, hormone-like substances used in tenderizing poultry, are not necessary to production. Accordingly, such drugs may be used only when it can be shown that no residues remain in edible products. The following is quoted from the Administration policy statement that was published in the *Federal Register* on December 4, 1948: "In considering a new-drug application for a product intended to effect physiological changes in farm animals, the Federal Security Agency (now the Department of Health, Education, and Welfare) will regard the absence of satisfactory evidence showing that the meat or other food obtained from animals fed the drug is entirely free of any poisonous or deleterious ingredient resulting therefrom at the time of marketing as ground for refusal to make the application effective."

In a sense this position rests legally on section 402 (a) (2) and 406 of the Act. These define as adulterated, a food, for example, poultry, containing any amount of an added poisonous or deleterious substance, such as diethylstilbestrol, which is not required in its production. Most of these articles being used to affect the physiologic function of livestock and poultry are regarded as new drugs. This requires submission of adequate evidence of safety for the animal or fowl and the absence of any residues in edible products prior to commercial distribution.

Now that we recognize the types of safety that a new drug application must establish whether it is intended as a therapeutic agent or to affect the physiologic function, we may ask "What kind of data are needed?" A categorical answer cannot be given. The data, of course, must be characterized by scientific accuracy,

comprehensiveness, reproducibility, and those other factors that are inherent in a well-planned, scientifically controlled study. An application may be refused unless it includes adequate tests by all reasonably applicable methods to show whether or not the drug is safe and unless the results of the tests show that the drug actually is safe.

In our consideration of a new drug application we are concerned primarily with the experimental data to show whether or not the ingredients, individually and in the final combination, are safe when used as the labeling directs. Please note the phrase "when used as the labeling directs," since this is important in our consideration not only of the safety of the drug to the animal but of the safety of the treated animal for human consumption. There is also the very real problem of suitable disposition of the by-products of slaughter so that organs wherein residues of the drugs may lodge will not harm animals to which they may be fed.

Where there are already available, through published scientific articles, ample factual data to establish the safety of a drug, copies of such articles may be sufficient for the submission of a new drug application from the standpoint of safety of the drug when used according to the directions in its proposed labeling. In the event that a drug has already been extensively used under practical conditions for a considerable period of time and there is no longer any question as to its safety when so used, it may no longer be a new drug as defined by the Act.

We are also definitely concerned with the methods used in the manufacture, processing, and packing of the drug and the facilities and controls used to determine its identity, strength, quality, and purity. This has an important bearing on the safety of the drug when used as directed and, together with the experimental evidence showing the safety of the drug, is given careful consideration.

Some manufacturers and distributors of drugs have the erroneous belief that if a new drug application filed by one manufacturer is effective for a particular drug, others who wish to manufacture the same drug do not need to obtain an effective new drug application. There is nothing in the Act that justifies this belief. It is the responsibility of each manufacturer or distributor to obtain an effective new drug application for his own product if it is a new drug by statutory definition.

The manufacturer of a drug for which a new drug application has become effective may wish to assist others to manufacture the same drug under their own labeling. He may therefore authorize the Food and Drug Administration to use his experimental data relating to safety along with the manufacturing and control methods used to establish its identity, strength, quality, and purity in behalf of the new applicant. All other requirements of the Act for the submission of a complete new drug application must be fulfilled by each applicant.

If any material change is made in formulation of the product, the original experimental data or safety may no longer be applicable to the drug and therefore additional safety studies may be required.

A manufacturer who has an effective new drug application may wish to sell his product to distributors for resale under their own labels. Under such circumstances, a supplement to the application is required. Such a supplement should include a statement from the manufacturer showing that he will supply the drug described in the application to the distributor with the distributor's labeling, specimens of which are attached. It should also include a statement from the distributor showing that he will distribute the article only under his labeling, speci-

mens of which are attached. Revisions in the labeling may be provided through additional supplements.

It has become a common practice for the primary supplier of a new drug substance to make it available to feed manufacturers in the form of a premix intended for use in the manufacture of a finished medicated feed. A new drug application may be allowed for a such a premix provided that its manufacturer agrees to limit its sale to feed manufacturers who have an effective application for the finished article. The finished feed is a new drug under these circumstances as long as the premix is a new drug. An effective application is required for it even though its distribution is confined within a state, if the premix is obtained in interstate commerce. In situations of this kind it is helpful to the premix manufacturer, to the medicated feed manufacturer, and to the Food and Drug Administration, which must consider each application or supplement, if a sound and uniform pattern of application is adopted, including specifications covering composition, manufacturing, and control provisions, and labeling acceptable to State Feed Control Officials. The adoption of such a scheme of operation has been found greatly to facilitate the early availability of new drugs on a wide scale. A similar pattern of operations is applicable to medicated feeds containing certifiable antibiotic drugs, which exempt them from the new drug provisions of the Act.

By the time a new drug application has been made effective, there may have been changes in the composition, methods of manufacture, controls, or labeling of the drug to insure its safety and integrity. Such insurance can be sound only as long as the manufacturer recognizes and observes the conditions of the application. The conditions of an application may be revised by supplementing an application whenever indicated. No such formality is required after an article ceases to be a new drug. But the unilateral revision of the conditions by a manufacturer may not only furnish grounds for suspension of an application but may threaten public health or give a black eye to a drug or an industry.

The widespread use of feeds as vehicles for the administration of medication has clearly put feed manufacturers into the drug business. Surely, we can all agree that the future prosperity and well-being of the feed industry depends on its meeting its responsibility to conduct its drug business in the public interest, especially in matters affecting the public health. This responsibility extends not only to properly controlled production of medicated feeds that are safe and efficacious when administered to farm animals, but also to the safety to consumers of the edible animal products affected by the medication. No residues of poisonous or deleterious substances in such edible products are permitted by law unless the drug is necessary to production. If the drug is needed, and we do recognize the need to use drugs to prevent or treat diseases of livestock and poultry, then residues are permitted in amounts that are safe.

The medicated feed industry represents a highly successful business. I am one of those who believe, and not without personal knowledge, that it recognizes its responsibilties in the matter of protecting the public health. From my own experience with its representatives in relation to this particular area of operation, I am hopeful that we can continue working together in the common enterprise of furnishing the American livestock and poultry farmer with the best and safest drugs that are humanly possible to make.

Description of the Methods Used in, and the Facilities, and Controls Used for the Manufacture, Processing, and Packing of Medicated Feeds (505[b] [4])

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The rapid increase in the development and use of medicated feeds has created many problems in the proper control of these articles. Among other problems, medicated feed manufacturers occasionally find on the submission of new drug applications that their concepts of adequate control differ from those of the New Drug Branch.

These differences can be reduced by a better understanding of the purpose of section 505 of the Federal Food, Drug, and Cosmetic Act. This section of the Act is intended to insure that new drug products are safe. What does a demonstration of the safety of a drug entail? It requires, in addition to investigations proving the safety of particular batches of material, a showing that all subsequent batches of the drug to be made will be identical to those for which safety has been established.

Section 505 (d) (3) of the Act requires that, to obtain an effective new drug application, the application must show that the methods used in and the facilities and controls used for the manufacture, processing, and packing of the drug are adequate to preserve its identity, strength, quality, and purity. This requirement gives the meaning to the provision of section 505 (b) (4) of the Act that a new drug application should include a full description of the methods used in and the facilities and controls used for the manufacture, processing, and packing of the drug. Apparently such information should be sufficiently detailed and complete to enable the New Drug Branch to evaluate whether or not all batches will be uniform in identity, strength, purity, and quality, and, therefore, safe.

Paragraph 4 of the new drug application form is a practical guide in preparing a full description of manufacturing methods, facilities, and controls. It is designed to assist the applicant in preparing a satisfactory submission but can succeed in this only to the extent it is understood and used. The purpose of this paper is to discuss its meaning.

We recognize that the adequacy of the manufacturing methods, facilities, and controls described in each application must be evaluated on its own merits. The very nature of this discussion precludes any series of categorical or precise definitions. In the following discussion we have given consideration to the fact that we

cannot at this time reasonably expect medicated feed manufacturers to employ the kind of control commonly found in the pharmaceutical industry.

Section (4) (a) of the new drug application form states that a full description of the methods used in the manufacture, processing, and packing of the drug be furnished. Just what does this embody? If the drug constituent of the medicated feed is a compound for which there are no official or recognized standards, or the manner of its preparation is not generally known or accepted, the methods used in its synthesis, extraction, isolation, or purification would be required. Furthermore, this description should include, in sufficient detail, such factors as time of reaction, temperature, *p*H, and solvents, to the extent necessary to establish the identity, strength, quality, or purity of the drug and the adequacy of specifications and laboratory tests. These descriptions usually can be adequate with less than full disclosure of "technical know-how" or trade secrets. We are interested in the identity and purity of the drug product. We are interested in its purification to eliminate solvents, catalysts, by-products, and other possible deleterious materials. For example, let us take diethylstilbestrol, since we are all familiar with its current use in feeds. Since this article is official in the *United States Pharmacopeia,* applications for medicated feeds containing this drug would not need to describe its preparation, if the application provides for use of the USP material. In such a case, we believe that use of the official specifications and test methods is sufficient to insure the identity, strength, quality, and purity of this compound.

Under this same section, a description of the methods used in the processing and packing of the finished feed or premix is also required. It should show the order and manner in which the various components are mixed and the precautions taken to insure uniform distribution of the drug component.

In the event the applicant does not himself perform all of the manufacturing, processing, and packing operations from production of the new drug constituent to packing and labeling of the finished feed, the application should clearly show what firms perform each operation. In addition, each firm that performs a part of the operations is required to submit a full description of its part. Let us take a hypothetical example: A firm submits a new drug application for a medicated feed providing for Company A to furnish the premix concentrate and Company C to manufacture the finished feed for the applicant, and Company D to assay it. Since each company is responsible for an operation in the preparation of the drug, a statement would be required from each.

Section (4) (b) of the new drug application form states that a full description of the facilities and controls used for the manufacture, processing, and packing of the drug is required. The form has listed beneath this caption specific points to be covered. We will discuss these points in the order in which they appear.

What should be furnished under a description of the physical facilities used in the manufacture, processing, packing, and control operations? Such equipment as the mixers, with their capacity, and packaging machinery should be described. In this regard, the application should state whether continuous or batch processes will be employed. Flow sheets would augment this description and are to be recommended.

In regard to the precautions taken to insure proper identity, strength, quality, and purity of the raw materials, the emphasis in the case of medicated feeds certainly should be placed on the active components. The customary feed com-

ponents recognized in the *Official Publication of the Association of American Feed Control Officials,* such as cottonseed or soybean meal, should preferably be checked for compliance with the specifications of this publication. In the case of a drug ingredient not generally used in feeds, the application should contain adequate specifications and laboratory test methods to insure its identity, strength, quality, and purity. The adoption of official standards and methods for a compound recognized in the official compendia would usually suffice in this regard.

It is the usual practice in drug manufacturing to assign a serial number to each lot of raw material, so that a history of each batch of finished product is available. This practice is desirable, but it is recognized that such control is not the customary practice of feed manufacturers. However, each lot of the active drug component, at least, should be identified in this fashion and the serial number noted on the formula card. In this manner each batch of the product can be identified with a specific lot of the active ingredient.

The application should describe the method of preparation of the formula card and the manner in which it is used. This is sufficiently clear in itself, but these points might be considered. The persons preparing and reviewing the formula card should be identified. If a master formula card is prepared and copies used for production, this should be stated. In this regard, photocopying is to be preferred over written transcription.

As to the number of individuals checking the weight or volume of each individual ingredient entering in a batch, we recommend that the active ingredients be checked by at least two responsible persons. Whether or not the total weight of a batch is determined at any stage of the manufacturing process, and by whom, presents problems inherent in feed manufacturing practice. For the major part, the large amounts of material handled may preclude the consideration of such a determination. But, on the other hand, consideration should be given to checking the total number of finished packages, or sacks, produced per batch with the expected theoretical yield.

The precautions taken to insure that the proper labels are placed on the finished feed or premix for a particular lot is self-explanatory and requires no additional comment except that such information should include the provisions for label storage and inventory control.

The importance of the next topic, the analytical controls used during the various stages of the manufacture, processing, and packing of the medicated feed, is self-evident. This information should include detailed descriptions of the collection of samples, the analytical procedures to which they are subjected, and the specifications required for acceptance of each lot of the finished article.

The analytical method used in each case should be described in sufficient detail to permit its duplication in our laboratories. If either official or published methods are used, specific citation of the literature will usually suffice. However, if a modification of the published method is made, the change should be described.

The proposed limits of assay of a premix should be held to as narrow a range as is ordinarily required of any drug preparation. However, the limits of assay on a finished feed may have a wider range. For instance, a certain type of premix is held to 98 to 108 per cent of label claim, while the active drug content of the finished feed in which the drug is incorporated has a range of 90 to 110 per cent.

The specifications for the active drug content of the premix or finished feed will be based on, and reflect, the adequacy of the assay procedure. As example, specifications for metals or inorganic compounds, like cadmium or arsenic trioxide, can reasonably be held to narrow limits, while specifications for some organic compounds may have wider ranges.

If the stability of the active drug is not known, stability studies should be performed and the results included as part of the new drug application. When such tests indicate that the drug may be unstable, an appropriate expiration date or outdating period should be proposed in the new drug application.

We hope that this discussion will assist you in the preparation of section 4 (b) of a new drug application and that better understanding of this topic has been achieved.

Design of Medicated Feed Supplements

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In incorporating a medicating agent in a feed, it is important that all the medicating agent be in the batch of feed to which it is added and that it be uniformly distributed. A physical loss of drug is a direct loss to the feed manufacturer, who must add more material as compensation. Even if most of the lost drug is recovered as product from the dust collector, it is unlikely to be returned to the batch for which it was originally intended, and it may create a problem when the feed manufacture is shifted to a different type in which the medicating agent is not desired.

It is obvious that uniform distribution of a medicating agent in feed will produce more satisfactory results than those obtained from feed containing an erratic distribution of medicating agent. With grossly inadequate mixing, there is a possibility that part of the feed will contain so little medicating agent as to be ineffective or so much medicating agent as to be toxic.

Poor mixing raises a second problem. Under such conditions, an ingredient added in small quantities frequently will not show a regular pattern of distribution but will tend to give a median value lower than the calculated average. This is probably due to the fact that a part of the ingredient will remain undispersed, forming small zones with localized high concentrations. Under such conditions of poor mixing, the feed manufacturer is further penalized, since he must add more drug to insure meeting the guaranteed medicating agent content of his feed.

In figure 1, curve A illustrates the uniform distribution of a microingredient in feed, while curve B illustrates uneven distribution. Curve B is not only less sharp than curve A but has a skewed shape. The difference between the peaks of curves A and B represents the extra microingredient that must be added to compensate for poor mixing.

Most medicating agents today are marketed as supplements rather than as 100 per cent active drugs. This practice provides an opportunity for the chemical manufacturer to cooperate with the feed manufacturer in insuring proper distribution of the drug in the feed by providing the medicating agent in the form of optimal physical properties conducive to good mixing and convenient handling.

In this review a distinction is made between the terms "supplement" and "premix." By supplement is meant the product sold by the chemical manufacturer, which may contain as much as 25 per cent active ingredient. By premix is meant a dilution of the supplement containing perhaps 0.5 to 5 per cent drug. This

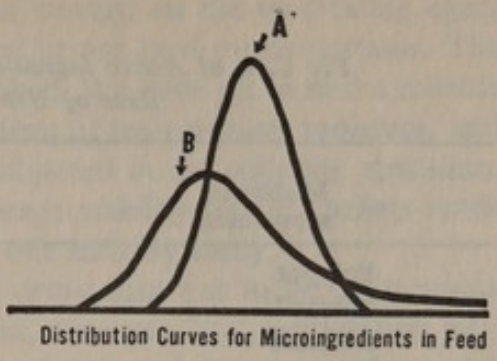

FIG. 1. Distribution curves for microingredients in feed.

premix is prepared by the feed manufacturer from the feed supplement. It is recommended that such premixes be prepared to insure good distribution of the drug in the feed. While in isolated cases a feed manufacturer may be able to mix the supplement directly into his feed, such cases are uncommon and probably indicate the feed manufacturer is employing a mixing intensity greater than would be required if he were to use a premix.

What elements must be considered in designing a good supplement? Several factors must be considered by the formulator; some of these are so obvious as to require only brief consideration. Some are so basic they cannot be qualified. Other factors represent ideals that can at best be approached only by sacrificing some other important property, and it is therefore necessary for the formulator to effect a compromise in balancing the desirable features of one factor against others.

FEED SUPPLEMENT

Concentration. Perhaps the first factor to be decided in designing a feed supplement is the concentration of the active ingredient; and here the first point of compromise is reached. The feed manufacturer in general prefers a dilute supplement, since the more dilute the supplement the more readily it can be made into a premix and incorporated into a feed. On the other hand, the chemical manufacturer tends to prefer a concentrated supplement in order to minimize the cost of accessory materials and shipping charges. On this question, the formulator is on the side of the feed manufacturer since, as a rule, the more dilute the formulation, the more successfully can the desired physical properties be incorporated into the supplement.

One further point that frequently determines the concentration of the active ingredient should be mentioned. It has become the practice to select a concentration such that 1 or 2 lb., or perhaps even 5 lb., of supplement represents the amount needed to medicate 1 ton of feed. Thus, in a case where 1 Gm. of medicating agent is required per ton of feed, one might try to design a supplement containing 1 Gm. of medicating agent per pound. On the other hand, a coccidiostat used at a level of 0.0125 per cent, or one part in 8000, would suggest a concentration of 25 per cent or, perhaps, 12.5 per cent in the feed supplement, corresponding to a use level of 1 or 2 lb. of supplement per ton of feed.

Table I shows a list of several commercial supplements, illustrating the trend toward use of round numbers in either the proportion of active ingredient in the supplement or the rate of use of the supplement in feed.

TABLE I

Per Cent of Active Ingredient in Several Commercial Supplements and Rate of Use of the Supplements in Feed

Medicated supplement	Total per cent active ingredient	Supplement added/ ton of finished feed, lb.
Polystat	35	4
Nitrosal	39	2
Megasul	25	1.5–2
NFZ	11.2	1
Nicarbazin	25	1
Sulfaquinoxaline–25%	25	1–2

Stability. The problem of stability must be faced by the formulator at an early stage in his study. One of the reasons for this is that knowledge of the stability of an ingredient in feed is dependent upon having a suitable method for the determination of this ingredient, which may be more difficult and more time-consuming to develop than the other aspects of the formulation study.

An understanding of stability requires a method of assay suitable for determining the active ingredient under conditions and concentrations of use. It is difficult to overstress the importance of having a good assay. It has been our experience that the extra time spent in refining an assay procedure so that it is more rapid or more convenient or more precise will be returned manyfold in savings during the stability testing program.

Chemical, microbiologic, and biologic analyses may all be used to establish stability. In general, we prefer chemical methods because they are more rapid and more precise, but a word might be in order here about the importance of having an assay that is specific for the material being tested. This is particularly a problem when chemical analyses are used. Thus an arsenic assay is of little value in establishing the stability of an organic arsenic derivative in feed, although once stability has been established, an assay for arsenic may be very useful in following the distribution of the arsenic derivative in feed. One must make sure that the chemical method of analysis is specific for the compound as a whole or for those groupings within the compound that are likely to suffer from instability. For this reason, we consider it advisable to run concurrent biologic and chemical assays in any stability program.

The chemical manufacturer must not only be concerned about stability of the drug in the supplement that he proposes to sell, but he must also be informed as to the stability of the drug in mash, in pellets, in mineral mixes, and in other multicomponent mixtures in which his medicating agent is likely to be used.

We have found that much useful preliminary information can be obtained in the laboratory stage of development by running accelerated stability tests, for example, for two months at 38 C. or one month at 50 C. on a mixture of the medicating agent in feed. For this purpose we prefer a feed with a relatively high moisture content, such as around 12 per cent, and fortified heavily with minerals to accentuate any sensitivity to oxidative decomposition. Such tests are likely to spot stability problems at an early stage in the development of the formulation; but

it should be emphasized that they are never a substitute for stability tests conducted under normal or mildly accelerated conditions of storage on the medicating agent in mixtures of the type where it is to be used and at use level concentrations. The effect of pelleting on stability must not be overlooked. We have yet to find a reliable way in the laboratory to approximate the conditions of temperature, moisture, and physical stress to which a formulation may be subjected in the pelleting operation. It has therefore been our practice to conduct storage stability tests on pellets made in a commercial feed mill as an integral part of our stability study.

It is fortunate for the formulator that most drugs turn out to be quite stable under conditions of use. It should be pointed out, however, that formulation provides an opportunity for stabilization through use of such techniques as *p*H control, antioxidants, metal inactivators, and separation of incompatible ingredients by particle coating techniques.

Efficacy. Before the design of a medicated feed supplement has passed the laboratory stage, it is important that the formulation be rechecked to make sure that the efficacy of the active ingredient has not been impaired. Impairment in efficacy is uncommon, but it can happen if the particle size of the drug or its mode of absorption is critical or if particle coating techniques have been employed in order to solve a stability or compatibility problem. A biologic assay must be employed.

If the medicating agent is used in feed at extremely low concentration, one must make sure that there are enough particles present to provide adequate distribution when one is dealing with the quantities of feed likely to be consumed by very young animals, e.g., the very young chick may consume as little as 5 Gm. of feed per day. As a general rule, we feel that the particle size of the medicating agent should be such that in the finished feed, every gram of finished feed should contain at least two particles of drug.

Accessory Ingredients. The choice of accessory ingredients going into a medicated feed supplement is in part determined by cost and availability. The ingredients should be such that their use will raise no problem with respect to palatability or tolerance. It is wise to confine one's choice to ingredients that are accepted for feed use. It is essential to avoid those ingredients that have the reputation of being feed adulterants or are considered undesirable for feed use. Although only a minute amount of some of these accessory ingredients would be present in the finished feed, there may be regulations against the use of these. For example, we have found corncob meal and sugar cane begasse to have some very interesting properties with respect to absorption of liquids, but we feel that neither of these ingredients would be appropriate in our feed supplements because of state regulations against their use.

PHYSICAL PROPERTIES

Modification in the physical properties of the feed supplement is the area that provides the formulator with the greatest possibilities for contributing in a positive fashion to the ease and convenience in handling a medicating agent. Two basic types of processes are available to the formulator for incorporating a medicating agent into a feed supplement.

The dry process simply consists of preparing a dry mixture of the active in-

gredient with one or more diluents or carriers. This is usually the least expensive and simplest type of formulation process, and in many instances a highly satisfactory supplement can be prepared by this method.

The wet process may take several forms, such as the preparation of a solution of the active ingredient, which is absorbed on an insoluble carrier. An alternate method is the preparation of a suspension of the active ingredient that is applied as a coating on the surface of the insoluble carrier using a soluble adhesive as binder. Both processes involve subsequent drying of the mixture and grinding to produce a product in the desired mesh range. Although the wet process is more lengthy than the dry process, it is particularly suitable when one is dealing with hygroscopic materials, formulations with a high content of active ingredient, or when a very close control over the physical properties of the supplement is needed.

Combinations of these two processes may, of course, be used. One common combination is the preparation by wet process of granules of an active ingredient, which are then diluted by a dry mixing process.

A dry mixture of sulfaquinoxaline and distillers' dried grains gives a product that is dusty and from which the sulfaquinoxaline may separate. A solution of sulfaquinoxaline in sodium hydroxide, absorbed on corn distillers' dried grains, on the other hand, yields a product distinctly superior in physical properties and similar to the grains in physical appearance.

Dustiness. The ideal feed supplement should not be dusty. Dustiness of the feed supplement is an annoyance to the man who handles the supplement in the feed mill and may be a potential health and safety hazard. From that point of view of the finished feed, there may be a serious loss of the medicating agent to the dust collector, which may occur when the feed supplement is charged to the blender or when the medicated feed is conveyed to the storage bin. Although the dust collected in the dust collector may eventually be returned to the feed, one has no guarantee that it will be completely returned to the same batch of feed from which it originated. In present-day practices where a feed mill may shift back and forth several times a day between the manufacturer of medicated and non-medicated feeds, dustiness of the drug increases the possibility of carry-over of the medicating agent into feed mixtures where it is not intended to be present.

In the laboratory we have found it convenient to measure dustiness in a semiquantitative fashion by pouring the feed supplement out in a stream through a funnel in the presence of a horizontal air current. A measure of the amount of material lost as dust can be obtained by weighing the material recovered below the funnel. A comparison of the concentration of active ingredients in the recovered material with that of the original material gives an indication as to whether the medicating agent is stripped preferentially from the supplement during this type of treatment. We have found this laboratory test very useful in developing feed supplements. It is, however, no substitute for runs carried out in commercial mills where a material balance can be established around the quantity of medicating agent charged, the quantity of medicating agent recovered in terms of medicated feed, and the concentration of the medicating agent in the product from the dust collector.

In formulating by dry process, dustiness can be reduced by increasing the particle size of the drug, by reducing the concentration of drug in the supplement, and by selecting a carrier that has a high powder-retaining capacity. Corn dis-

tillers' dried grains and wheat middlings are examples of carriers with a high capacity for retaining powder, while soybean meal is an example of the carrier that in our hands has shown a low powder retention.

A more complete elimination of dust can be achieved by resorting to wet processing. Thus the medicating agent may be dissolved in a suitable solvent and absorbed onto a porous carrier or adhesion to the carrier may be achieved by employing a suspension of the medicating agent together with an appropriate adhesive. Sulfaquinoxaline – 25%, is an illustration of a wet process type of formulation prepared by absorbing a solution of the soluble sodium salt of sulfaquinoxaline onto corn distillers' dried grains.

Segregation. A second important physical property desired in the medicated feed supplement is freedom from segregation. Three types of segregation can be distinguished: segregation of the carrier, segregation of the active ingredients through sifting, and segregation of the active ingredients by the development of electrostatic charges.

Segregation of the carrier is encountered when the mixture is highly free-flowing or when the carrier itself contains too wide a range of particle size distribution or density variation. The segregation of bran from Red Dog flour is an illustration of this type of separation. It can be prevented by screening the bran from the flour or by milling the entire product in such a fashion that the bran particles are ground up.

More serious to the formulator are the effects of segregation of the active ingredients, since this can lead to a loss of control over the amount of drug added to a feed and can increase the carry-over of drugs from a batch of medicated feed to a following batch of nonmedicated feed.

The tendency of the active ingredients to separate from the carrier by sifting can be detected in the laboratory by putting a sample of the supplement on a shaking machine for 15 or 20 minutes and then running analyses on the top, center, and bottom portions of the supplement for active ingredient content. A disparity in the active ingredient content greater than that found in the original mixture indicates segregation. A more practical segregation test might consist in shipping a 50 lb. bag of supplement several hundred miles by truck and running similar analyses on different portions of the product.

In supplements prepared by a dry process, segregation of the active ingredient can be reduced by increasing the particle size of the active ingredient, by reducing the percentage of active ingredient in the supplement, and by selecting a carrier with high powder-retaining capacity and with the proper particle size. Segregation through sifting is usually encountered with mixtures that are very free-flowing, and, if necessary, one can reduce the tendency to segregation by reducing the free-flowing properties of the mixture by the addition of a small quantity of vegetable oil or by decreasing the particle size of the carrier.

Formulations prepared by wet processing in which the active ingredient is absorbed in or is adhered to the carrier usually are free from any serious segregation.

Medicating agents that have been milled to fine powders and are highly insoluble in water may show a tendency to acquire electrostatic charges. In many cases, a formulation of such an ingredient can be handled quite satisfactorily as long as one is dealing entirely with metal equipment. However, when such supplements

are placed in contact with nonconducting materials, such as fiber or paper containers, plastic, glass, and rubber utensils, the active ingredients may separate from the supplement and adhere to these materials in a thick layer that is quite difficult to remove. Such electrostatic tendencies can lead to appreciable losses of active ingredients during feed manufacture and will increase the tendency of medicating agents to carry over into batches of feed that should have no medicating agent.

The tendency of medicating agents to develop electrostatic charges is by no means entirely disadvantageous. Feed supplement carriers themselves are ordinarily not conductors of electricity, and a tight electrostatic bond that will act like an adhesive in holding the medicating agent in place can be established between the medicating agent and the carrier. A common method for reducing the tendency of the medicating agent to cling to nonconducting equipment by electrostatic bonds is simply to dilute the supplement to the point where there is sufficient carrier available to compete with such equipment in attracting the medicating agent.

The tendency of a medicating agent to develop electrostatic charges can be reduced by applying a small quantity of a surface active agent or humectant, like sorbitol or glycerol, to the surface of the medicating agent. Application of these materials can frequently be carried out conveniently by applying them as wash to the medicating agent during the last step of manufacture, prior to drying. Wet process techniques for preparing feed supplements usually eliminate the electrostatic problem with medicating agents.

Flow Properties. Free-flowing properties are particularly important in a feed supplement to be mixed in feed manufactured by a continuous process. In this case the feed supplement or a premix prepared from it is proportioned through a machine onto a conveyor belt on which other ingredients to the feed are also proportioned. For controlled regular operation, it is necessary that the feed supplement pass through the proportioning machine freely and without the occurrence of clogging.

The angle of repose and the flow of the feed supplement through a restricted opening represent laboratory procedures for judging flow properties. A more decisive test is to pass the supplement through one or several types of proportioning machines of the type used in the commercial continuous production of feeds.

In the preparation of feed supplements by dry blending techniques, flow properties may be improved by selecting a carrier with good flow properties itself, like soybean meal, and by selecting a particle size distribution of the carrier intended to give the best flow properties. It should be noted that as the free-flowing properties of the dry mixture increase, the tendency of active ingredients to segregate from the carrier will likewise increase. Therefore, it is necessary for the formulator to effect a balance between these two factors.

The addition of small quantities of so-called free-flowing agents to formulations frequently effects a marked improvement in flow properties. These agents may be used in amounts up to about 5 per cent, although significant improvements may be noticed with as little as 1 per cent free-flowing agent. Some of the more effective free-flowing agents include calcium silicate, magnesium trisilicate, calcium phosphate, and talc.

In wet process formulations where the active ingredient is firmly affixed to

the carrier, concern with segregation of the medicating agent in developing a free-flowing mixture is less acute.

Caking and Packing. The tendency of a feed supplement to cake or pack under conditions of use is a property that must be considered. By caking, we mean the tendency of a mixture to form hard lumps, either through uptake of moisture or storage under pressure. A caked feed supplement poses a serious handling problem for the feed manufacturer, for unless the lumps are broken up very carefully, small lumps of the feed supplement may survive in the finished feed. By a packed supplement, we mean the tendency of a feed supplement to increase in apparent density during shipping so that it is no longer free-flowing. While the lumps which form in the packed supplement represent a nuisance to the feed manufacturer, they usually break up fairly easily and do not survive in the finished feed. The tendency of a feed supplement to pack can lead to erratic feeding if the supplement is fed through a proportioning machine used in the manufacture of feed, since a packing of the supplement, which is, in effect, a change in density, can seriously disrupt the rate of feeding.

The tendency of a supplement to cake can usually be spotted in the laboratory by storing the mixture for several days at about 100 F. and 85 per cent humidity. Following this treatment, the mixture is transferred to a 60 C. oven and dried without disturbing the supplement. A good supplement will survive this treatment and remain free-flowing throughout. A supplement showing a tendency to cake through hygroscopicity will emerge from this treatment as a hard, solid lump. The tendency of supplements to cake is accentuated by pressure, and the pressure applied to a bag of medicated feed supplement, which may be located on the bottom of a number of vertically stacked pallets, perhaps 10 to 15 bags high, is quite appreciable. As a laboratory test, we sometimes employ the technique of placing a cloth bag of supplement between the jaws of a vise and storing it several days in this fashion. An alternate procedure is to take a small cloth bag of supplement and place it under a stack of feed supplement pallets.

The tendency of a feed supplement to cake is ordinarily due to the hygroscopic or low melting properties of the ingredients. In supplements prepared by a dry process, this can be reduced by minimizing the quantity of medicating agent in the supplement, by selecting a carrier, like distillers' dried grains, which has strong absorptive properties, and by the use of free-flowing agents like calcium phosphate, calcium silicate, and talc.

The tendency of a feed supplement to cake can also be overcome by resorting to a wet process for manufacture. Thus a choline chloride supplement prepared by absorbing a concentrated aqueous solution of choline chloride on distillers' dried grains, followed by drying, shows much less of a tendency to cake than a dry mixture of choline chloride crystals and distillers' dried grains.

Particle Size and Mesh. In anticipating that the medicated feed supplement is most likely to be mixed with a grain carrier in the preparation of a premix by the feed manufacturer, it is important that the feed supplement be generally compatible in particle size and mesh.

It has been our experience that a supplement all through 20 mesh, practically all through 40 mesh, and a minimum through 200 mesh is preferred. Simple laboratory screen analysis is sufficient to establish mesh distribution.

We have obtained satisfactory results with supplements varying from 25 to

75 lb./cubic foot in bulk, although a bulk close to 35 lb./cubic foot is probably preferred. Bulk is rarely a problem in dry process supplements prepared with grain carriers but may require greater consideration in wet process supplements, which tend to run higher in density.

SUMMARY

We have attempted to list some of the important factors that must be considered in designing a medicated feed supplement. Concentration of active ingredient, stability, efficacy, and acceptability of accessory ingredients must be established, while a satisfactory balance must be maintained among important physical properties, like dustiness, segregation, flow, caking tendencies, mesh, and bulk.

While the ultimate incorporation of the medicating agent into feed is the task of the feed manufacturer, careful design of the feed supplement can do much toward presenting the medicating agent in an attractive form that is convenient to handle and conducive to quantitative and uniform incorporation in feed.

The Determination of Drugs in Medicated Feeds

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The unprecedented increase in the diversity of nonantibiotic drugs sold in medicated feeds to the poultry industry the past few years has brought a corresponding increase in chemical problems to control laboratories engaged in checking drug content against label guarantee. Most of the difficulties stem from the lack of sufficient official methods for determining all the different drugs, of which about 22 are now used, and the complex, multidrug mixtures. Although seven authoritative methods are sponsored by the Association of Official Agricultural Chemists, more than three times that many should be available. The Association expects to meet some of the more pressing requirements this year, but, if the introduction of new drugs continues apace, a lag between desire and fulfillment will prevail.

Meanwhile, the Association has taken cognizance of current requirements by appointing six Associate Referees on Drugs in Feeds for methods development. Three represent industry, two are in state control laboratories, and one is in the Food and Drug Administration. If their plans mature and more methods can be adopted, the additional assay procedures will help considerably. However, since approval of a method depends on proof of accuracy obtained through collaboration, final adoption cannot take place until next fall at the annual Association meeting.

Association work exacts a considerable toll of voluntary time, and few referees have the ample time necessary for extended research. For this reason, particularly if they lack procedures originated by themselves, they depend largely on methods developed in the research laboratories of those who manufacture or sponsor the drugs. These are submitted to collaborative chemists in other laboratories if their own exhaustive trials prove the methods have sufficient merit.

This self-trial has revealed that some of the preliminary methods recommended by the drug manufacturers have inherent errors that must be eliminated before they can be subjected to collaboration, but most of them have sufficient accuracy for routine checking purposes where tolerances are liberal. Nevertheless, there is official concern in having standard Association methods in place of these. Industry, itself, has acknowledged their tentative nature by continuing research and by substituting better procedures or offering advice on significant changes.

The concern of control officials is ultimately legal in nature. Methods having

the legality of court sanction or those that can be established in court as authoritative are desirable whenever prosecutions for label violations or fee assessments for drug deficiencies are intended. Furthermore, checks of the same feed sample between manufacturer and control laboratory, when questions of wide guarantee deviation arise, should be based on the same method. Otherwise, misunderstandings may result that are harmful to both interests. For these reasons, attainment of a high degree of method accuracy is uppermost in the plans of Associate Referees. When their studies of existing methods result in desirable modifications that increase precision and possibly shorten technique, the time they have spent is of value to everyone concerned.

The drugs they are studying this year are the following: arsanilic acid (*p*-aminobenzenearsonic acid); diethylstilbestrol (a,a'-diethylstilbenediol); furazolidone [N-(5-nitro-2-furfurylidene) 3-amino-2-oxazolidone]; nicarbazin (4,4'-dinitrocarbanilide:2-hydroxy-4,6,-dimethylpyrimidine); nitrofurazone (5-nitro-2-furfuraldehyde semicarbazone); and organic arsenicals in general. It is hoped an existing method for dienestrol diacetate (3,4-bis [*p*-hydroxyphenyl]-2,4-hexadiene) can also be studied this year. If time permits, there will be collaboration on a method for 4-nitrophenylarsonic acid and 3-nitro-4-hydroxyphenylarsonic acid. A method for the latter drug is in a nearly completed research stage.

At present, four standard methods for determining the drugs in feeds are listed in the recently published 8th edition, *Official Methods of Analysis* of the Association. They are for sulfaguanidine (N^1-guanylsulfanilamide), sulfaquinoxaline (2-sulfanilamido-quinoxaline), 2-amino-5-nitrothiazole, and arsanilic acid.

Of these drugs, the first is no longer commonly used in feeds, though it bears the distinction of being the first drug so used. The second is partially yielding place to a newer drug, nicarbazin, and 2-amino-5-nitrothiazole has been superseded by 2-acetylamino-5-nitrothiazole. Even the arsanilic acid method will not survive. Although it gives precise results on normal feeds, the current practice of superfatting certain special feeds with tallow and pelleting them, which tends to prevent complete drug extraction, makes modification of the method necessary.

From the changing scene in drug usage, it is evident that some of our official methods are losing their prior importance. It is likely that prophylactic and therapeutic practices of the future may make some of them obsolete because of new medicinal concepts. Also, it is fundamental policy that official methods be reviewed whenever shorter or more accurate techniques suggest the desirability of change.

A recommendation that the arsanilic acid method be so reviewed was concurred in by the A.O.A.C. at the sixty-ninth annual meeting last October. Also, at that time, the Association adopted three other methods, for nicarbazin, nitrophenide (m,m'-dinitrodiphenyl disulfide), and sulfaquinoxaline and arsanilic acid in the same feed, which were published in the Feb., 1956, Journal of the A.O.A.C. These were adopted too late for inclusion in the new book. The nicarbazin method has already undergone an important change. By shortening assay technique and increasing precision of results, the new Associate Referee has satisfactorily met the somewhat negative criticism of the older method that it took too much time.

The year's program of methods study, if carried to fruition, will largely satisfy most of the more urgent requirements. However, it will meet requirements only for drugs added singly to feeds. It will not take care of the prevailing trend toward so-called "polystat" mixtures of two or more drugs in the same feed. With such

mixtures, the chemist is likely to encounter interferences between drugs that produce erroneous results if he uses an official method intended for one drug only.

These interferences cannot always be predicted without perception of how drugs will react in the presence of each other while being assayed. For example, certain general procedures are in use for evaluating drugs by reacting them with reagents for azo dye formation. Spectrophotometric absorption measurements of the intensity of color are used for sulfaquinoxaline, arsanilic acid, and several other drugs, but, because the dyes formed from sulfaquinoxaline and arsanilic acid both show maximum absorption at 545 mμ, a method based on dye formation alone will not distinguish between these two drugs. For this reason, a separation procedure for them had to be devised and has now become official.

In the same way, nitrophenide and arsanilic acid occurring in the same feed require a special technique to destroy arsanilic acid when assaying for nitrophenide. The arsanilic acid method, however, when applied to such a dual drug mixture, does not pick up interference from nitrophenide. This is because arsanilic acid will couple without preliminary reduction, whereas nitrophenide must first be reduced so its nitro groups become amino groups capable of color formation.

Drug interferences are being studied by Associate Referees and at the analytical laboratory of the Connecticut Agricultural Experiment Station. Procedures for avoiding these interferences will supplement official methods as they are gradually worked out.

Another type of interference, but of a nondrug nature, came to light at the Connecticut laboratory a few years ago. It was discovered that prolonged, alkaline feed hydrolysis, by splitting protein into its component amino acids, liberated a substance showing a tendency toward dye formation. The substance was finally identified as tryptophane, an essential amino acid occurring in all feeds, which produces slowly developing color when diazotized and coupled with N-1-naphthylethylenediamine dihydrochloride. In the presence of nitrophenide this foreign color gave erroneously high results by an early method for the drug.

Since that time, methods dependent on azo dye formation have been carefully developed to avoid tryptophane interference. This is done by preventing excessive feed hydrolysis, by rigid control of drug solution *p*H, and by limiting the strength of diazotizing reagents. As a result, official methods calling for color measurement can be relied on not to show tryptophane interference. Any overly confident feeling, however, that such official methods can be used without due care, with inadequate attention to details, will result in error.

First in importance in the determination of drugs in medicated feeds is the proper collection of a representative sample at the warehouse, based on withdrawal of portions from a predetermined percentage of bags in the lot of any one brand. Indifference here by short-cutting bag sampling may nullify all subsequent work. At least 1 lb. of feed, preferably 2, should be riffled or baffled into the sample container, and an original label showing the brand, the drug or drugs present, and the guarantees should be attached thereto. In the grinding room of the laboratory it must never be assumed that the gross sample is completely uniform. Careful mixing of the entire sample should precede grinding to pass a sieve having circular openings 1 mm. in diameter.

This extra care is necessary in processing samples of medicated feed. To the chemist accustomed only to regular feed analyses where components of the feed

are determined as moisture, fat, fiber, protein, and ash, this extra precaution may seem overly vigilant. But from now on, to the new drug chemist, the feed itself will constitute the inert ingredient; only the drug is the active ingredient. These differences, simple as they are, lie at the heart of medicated feed determinations. The tiny particles of drug scattered throughout the finished feed are outside the scope of gravimetric or volumetric analysis. The analyst must now turn to spectroscopic instrumentation to measure the 50 to 250 parts per million of drug involved. However, to do so accurately, he must have a sample in which reasonable and practical homogeneity is present.

The size of sample taken for drug determination is another important factor. From 2 to 10 Gm., depending on whether it is ground or mixed with a carrier and depending on its concentration of drug, are usually recommended. It would be desirable to standardize sample size, but it probably cannot be done for all drugs because of distribution troubles. Micronizing the drugs to minimum particle size before they are premixed might solve some of the difficulties of uniform distribution if electrostatic effects can be eliminated. Even the static charges sometimes present when one weighs feed on a laboratory balance on a cold day may have a dispersal effect on minute drug particles and affect sampling accuracy.

Other factors affecting precision are readily recalled. Until proved otherwise, the stability of drugs in the presence of feed will aways be questionable. Whether some drugs become bound to protein so that portions are irrecoverable because insoluble, whether they undergo change through self-oxidation of the feed, or whether they are altered somewhat by mold or fungus are possibilities. Age and rancidity of feed seem to be associated with loss. What happens to certain drugs weeks or months after they are mixed with feeds is a profitable field of research.

We do know that some feeds may show drug deterioration much more rapidly after they are ground. Assays immediately before and after grinding have revealed some slight loss. This decline, differing from drug to drug, indicates how necessary it is to proceed with determinations promptly. Samples permitted to lie around the laboratory in subsample jars of clear glass exposed to excessive heat or sunlight for a few weeks should not be used. If, after grinding, delays cannot be avoided, it is best to cold-storage the samples.

Another important factor having a bearing on precision of assay is the scrupulous observance of the time limits, volumes, strengths, and freshness of the reagents specified. Unintentional variations may contribute to error.

All present official methods depend on a spectrophotometer or photoelectric colorimeter, whether of the filter, grating, or prism type. They have different advantages, but, for extreme accuracy, instruments of the highest precision are preferred. Regardless of the type, however, the analyst should have some means of checking his instrument for normal operation. Although the Association methods specify reference to standard curves prepared from spectrophotometric readings of pure drugs for evaluating results of assays, it has been found good practice to run a standard along with the drug determination to eliminate the possibility of instrumental error. It serves as a constant check on wavelength variation and current irregularity.

These are the essential factors the chemist keeps in mind during determinations. If he has done much research on methods development for his own laboratory, he

is well aware of how important they are. He finds each method different, yet in time comes to recognize the thread of similarity running through all of them.

At present, the methods thus far adopted by the Association are not specific because, in general, they depend on reactions common to each. Only chromatography, with its clear-cut separation of drug in pure form, seems to offer the ultimate in positive identification, but chromatographic methods are usually too lengthy for the busy routine laboratory. Thus, research workers to date have chosen to consider two approaches: either drugs may be reacted to form color or they may not.

Compounds, such as the sulfonamides, having an aryl amino group that can be diazotized and coupled for dye formation lend themselves most readily to direct methods development. Reactions of this nature have been used for sulfaguanidine, sulfaquinoxaline, sulfamerazine, sulfamethazine, and sulfadiazine, as well as other sulfonamides.

Their ability to couple smoothly and quickly with N-1-naphthylethylenediamine dihydrochloride, the most versatile reagent now used, is of decided advantage for visual measurement of drug concentration by comparison with pure drug standards. Arsanilic acid will also couple readily in the same manner. Color that can be seen and evaluated by its density is dramatic evidence of the drug's presence and has a visual esthetic appeal.

When color cannot be produced by direct azo dye coupling at room temperature, it can be formed after diazotization, as in the case of nicarbazin, in hot solution with an agent such as sulfanilic acid or *p*-aminobenzoic acid. Drugs containing a nitro group, or groups, such as nitrophenide and 4-nitrophenylarsonic acid are first reduced to amino compounds for coupling. The production of a red complex of furan compounds with phenylhydrazine will probably prove to be the reaction preferred for furazolidone and nitrofurazone. Condensation reactions for dienestrol diacetate and diethylstilbestrol offer possibilities. Phenothiazine is brominated for color reaction.

The alternate procedure to color visibility, or the direct reading of absorbance of unreacted drug in the ultraviolet region of the spectrum, is the general method resorted to when all else fails. Working with a clear solution of the drug, freed from pigment and protein insofar as possible, in water or an organic solvent, the analyst can measure the amount of compound present by its absorption reading in relation to a standard. The present official method for 2-amino-5-nitrothiazole depends on such a procedure.

If anything, readings thus taken in the ultraviolet spectrum from 200 to 400 mμ tend to be more specific for the drug than readings of color complexes in the red spectrum from 400 to 700 mμ. This is because of the peaks of maximum absorbance in the ultraviolet spectrum are at greater variance as between drugs than are the colored compounds measured in the red region and based on the same coupling reagent. However, positive identification of any drug by present means of assay is questionable. With the possible exception of the Association method for nicarbazin, where the two molecules forming the drug complex are determined separately, the methods are not sufficiently specific.

This has an unfortunate aspect in toxicity investigations of viscera from chickens or turkeys believed to have died from an overdosage of drug. Unless the drug in question is known, as well as whether other drugs were present in the feed,

such toxicologic examinations, although they may reveal the presence of drug in liver or stomach contents, will not positively identify what it is. One can rely only on probabilities, helped somewhat by the type of drug reaction. It is hoped that someday a schematic, qualitative procedure for drug identification in such possible poison cases may be worked out. Such an orderly plan for sulfaguanidine, sulfaquinoxaline, 2-amino-5-nitrothiazole, and nitrophenide in feeds was in use at the Connecticut laboratory a few years ago until the present intricacies of drug variety and mixture so voided the procedure that it had to be abandoned.

For some years now, ever since the first commercial medicated feeds appeared in quantity in 1948, it has been a source of satisfaction to notice the more temperate and enlightened viewpoints of feed manufacturer and control official. The beginnings of the medicated feed industry, which is now so vast and diversified, were somewhat rough and troublesome to both interests. Earlier drug methods were more uncertain of accuracy, and blending operations were equally uncertain. A morass of mutual doubt of each other's competence threatened to restrain normal progress toward better relations.

However, that initial period of stress is long past, and for some time it has been the experience in Connecticut to record a higher percentage of medicated feeds coming closer to guarantee. The tolerance allowed is plus or minus 20 per cent, which is fair, considering all the unfavorable factors that may influence the feed from the blending operation to the final assay figure. For the past few years, 80 per cent of Connecticut feeds received for inspection have come within the tolerance limits.

Today's greater understanding of problems mutual to the feed industry and control officials has brought acute awareness of several essentials. The proper collection, preparation, and care of samples, and the use of official methods in drug determinations are the responsibilities of control laboratories. The careful blending of drug and feed with adequate labeling and proper storage are the equal, coordinate responsibilities of industry. Both have a mutual goal and both have gone a long way toward its attainment.

Manufacturing and Quality Control Problems with Medicated Feeds

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The feed manufacturing industry has made substantial progress, as measured by field performance in the past 10 years. Drugs have made a definite contribution, yet their increased use has presented the feed manufacturer with greater problems. This report deals primarily with production and control problems after a drug has been found suitable for field use. Extensive research by the drug and feed manufacturer must precede this phase. We would like to recommend that any new drug be released to feed manufacturers for experimental purposes from three to six months in advance of promotion and general release. If this were done, many of the research, manufacturing, control, and field problems could be solved before the compound is used generally.

The drug manufacturer should provide toxicity information on most commercial feed-consuming animals for each drug offered. This includes animals other than the one for which the drug is intended, since many types of feed are made in each plant. If trace amounts produce any detrimental results, adequate mill precautions must be taken to prevent contamination.

Most of the following comments are related to continuous mixing rather than to batch mixing. Continuous mixing represents a major percentage of commercial feed tonnage. The physical characteristics of a drug are of primary manufacturing importance. It must be accurately dispersed to meet the requirements for full effectiveness and to prevent overdosage, as well as to satisfy the legal requirements with respect to the guarantee. If used as a dry premix, granulation, the tendency to segregate, and any electrostatic properties are of paramount importance. Generally speaking, the concentration of drug premixes as they come from the pharmaceutical manufacturer should be sufficiently low to permit direct addition to the feed stream through a chemical feeder. For the commonly used drugs, the drug manufacturer should provide premixes ranging from 10 to 25 per cent in drug concentration. These can be added directly to feeds with the proper equipment without further premixing, if they have satisfactory flow characteristics.

Premixing in conjunction with a continuous mixing operation is costly. One of the primary responsibilities of the feed industry is to produce feeds at the lowest possible costs to the farmer. Each additional plant operation contributes to greater costs and should be avoided. An additional problem is the possible contamination of batch-blending equipment normally used for other purposes. For example, drug

contamination of dog food by use of the same equipment for making premixes and dog food has resulted in animal deaths. In our operations, batch mixers used for dog food are never used to premix drugs or other potentially hazardous compounds.

In our experience, the addition of drugs and other compounds in solution form by a pump-dispersion system is preferred to the use of dry premixes. Dispersion is excellent and accurate additions can be made consistently at levels as low as .025 per cent of a given solution, although a slightly higher use rate is generally preferred. Unfortunately, many of the commonly used drugs are neither soluble nor miscible in water or oil and must be added in premix form. A liquid medium avoids problems of dusting and minimizes electrostatic difficulties. Our experience with sulfaquinoxaline and arsanilic acid, both added in solution as the sodium salt, has been excellent. Incidentally, water-soluble or miscible vitamins in solution can be dispersed into mixed feed very nicely.

Two important principles should be applied to the addition of drugs or other critical trace ingredients, particularly if they are added in dry form. First, the location of the drug feeder on the collecting conveyor should be as close to the mixer as possible for best results. Second, the drug feeder should be located between the mixer and the corn meal and soybean oil meal feeders so these ingredients can carry the drug along and act as a pusher. Both the dry and liquid ingredients are added to the feed stream on a weight basis. Drives for all pumps and feeders are adjustable to different use rates. Recording is done by supervisors and control room men, but all records are checked by a supervisor.

Following the decision to use a drug, plant procedure must be set up. Mill test runs help determine the efficacy of mixing the new compound. Samples of the first and last bags in the run and samples taken at intervals throughout the run are desirable. In addition, samples taken at six points within individual bags of the run show the drug dispersion.

The number of pullouts or setbacks required can be determined from such studies. These are bags at the beginning or end of a run which may not contain the full amount of the drug. They can be a serious problem, since they cannot be run into other feeds, even at a low rate, without causing a serious contamination problem. Even very low levels of nicarbazin in laying or breeder feeds can adversely affect hatchability and egg shell color.

Our usual plant procedure is to pull off at the packer units the first 6 to 12 bags of a run and a similar number at the end. The exact number pulled is different for each plant. Chemical analyses are an important aid to the solution of this problem. The bags of feed removed from each run in this manner are added at a low rate into the same type of feed. Medicated broiler rations, for example, are added back to unmedicated broiler rations at a low rate. The shorter runs of feed magnify the problem, since a larger percentage of the bags packed would be pull-offs. As we use more drugs, pull-offs and manufacturing sequence become more critical.

Data from one of our first nicarbazin test runs are shown in figure 1. The preceding run contained sulfaquinoxaline and sodium arsanilate. The run is not an outstanding example of drug distribution but is reproduced to show what problems can be expected. No bags were set back at the start of this run or the following run. The data show that the theory level, .0134 per cent $\pm$ 10 per cent, was not reached until approximately 12 bags were manufactured. We allow the plants a

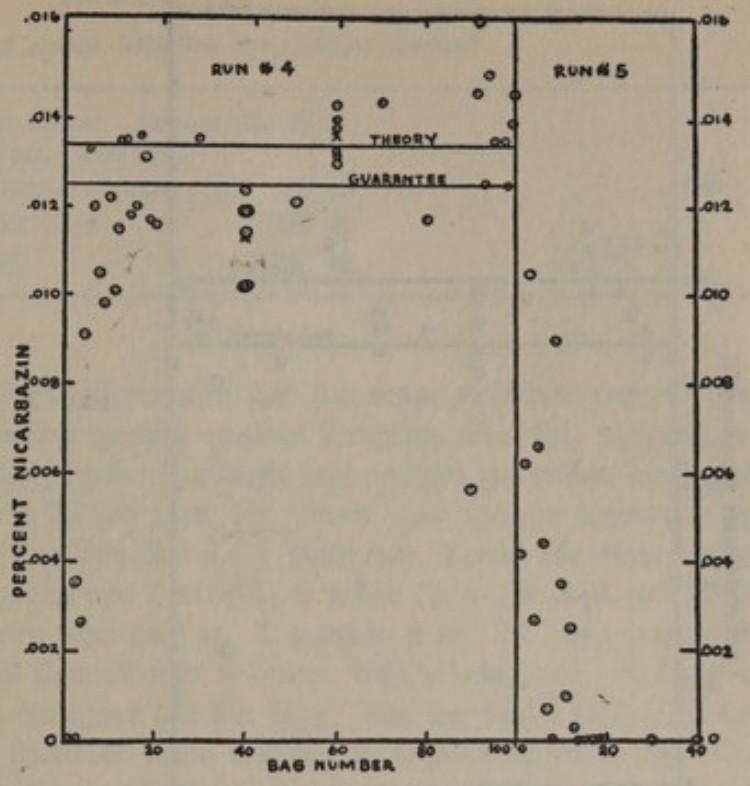

FIG. 1. Nicarbazin distribution run no. 4 and run no. 5

tolerance of a 10 per cent theoretical level, so for this run the low tolerance would be .0121 per cent. Bag numbers 40 and 60 were sampled at six points, the cross indicating the average of the six values. Bag 40 showed considerable variation and was below theory and guarantee. Bag 60, on the other hand, showed a desirable content and distribution of nicarbazin. An interruption in nicarbazin flow occurred at about the ninetieth bag in the run, accounting for the low value in this sample. Following this run, run no. 5 containing sulfaquinoxaline and sodium arsanilate was made, and residual nicarbazin was determined. Again, approximately 12 bags were made before a negligible amount of nicarbazin was found. A number of similar tests clearly indicate that a separate study must be made at each plant and for each drug.

Figures 2 and 3 show sodium arsanilate and diphenyl-*p*-phenylenediamine (DPPD) distribution in run no. 4. Both compounds were present in the runs preceding and following the one for which distribution charts are reproduced. Sodium arsanilate is added as a 10 per cent solution and DPPD as a 25 per cent

TABLE I

Twenty-five Per Cent Nicarbazin Production and Control Sheet

Lot no. V 6-431-5	Date manufactured August 22, 1955
Scale	Feed Improved Hi-Ener-G Nicarb Arsan
Start 660.5 lb.	Tag serial T 542109–T 544761
Stop 524.5 lb.	Cwts. made 2603
Used 136 lb.	Mill result .0130%

Laboratory analysis: no. 1, .0126%; no. 2, not run

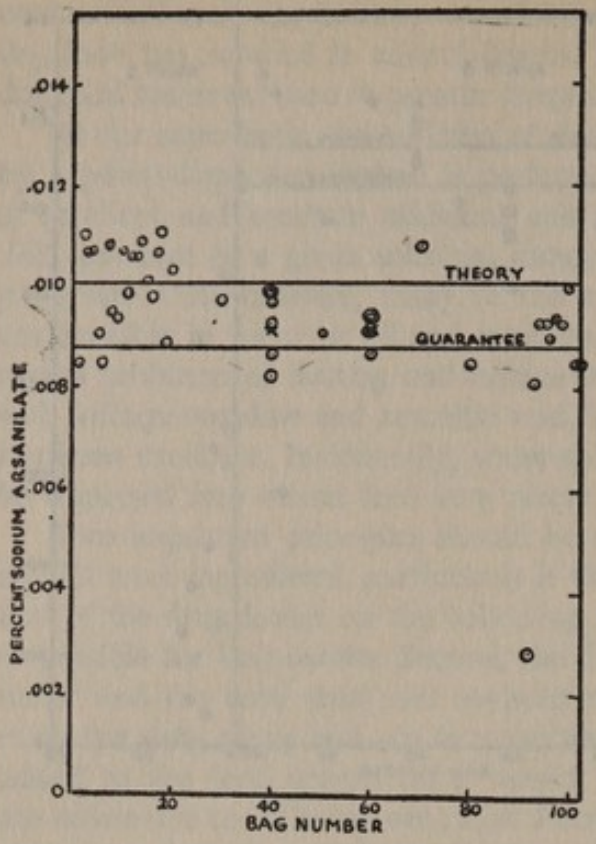

FIG. 2. Sodium arsanilate distribution in run no. 4.

dry premix. Again, the fortieth and sixtieth bag analyses are for six points within these bags and the crosses are the average of the six dispersion values. Run no. 4 actually involved 102 bags, and this figure was used on the DPPD and arsanilate figures, but only 100 bags are indicated on the nicarbazin figure. We do not have the same drug distribution pattern for these figures as for the nicarbazin plot because the preceding and following run both contained DPPD and arsanilate.

In the addition of all drugs to our feeds, we add a 5 to 10 per cent overage of each drug to cover any losses and to meet more effectively the guarantee. The overage will depend on the nature of the drug. The plants are held to ± 10 per cent of the calculated theory level.

TABLE II

Twenty-five Per Cent Nicarbazin Monthly Proof Sheet

On hand August 1, 1955	2269½	
Received during month	6800	
Total	9069½	9069½
On hand end of month	2617½	2617½
Inventory used, August, 1955		6452
Used by record sheets, August, 1955		6451
		1

Plus 1 lb.

TABLE III

Ten Per Cent Arsan Solution Preparation Record

Date mixed	August 22, 1955
Lot no.	666-7236
Sodium arsanilate used	40 lb.
Water used	320 lb.
Total	360 lb.

Tables I to VII indicate both the inventory and use data. Accurate records are extremely important in an effective quality control program. For this purpose, a 2603 bag run of a feed containing both nicarbazin and sodium arsanilate has been used. Nicarbazin was added as a 25 per cent dry premix and sodium arsanilate as a 10 per cent solution. Table I shows the daily plant use record for nicarbazin. In the routine control program, the no. 1 sample is taken from 20 bags scattered through the first half of the run and the no. 2 sample from 20 bags scattered through the second half. If the first sample is found within tolerance, ± 10 per cent, the second sample is not analyzed for the drug. The tag serial indicates the feed tags used for the run. A different serial number is printed on each tag. For each run of feed, the inclusive tag numbers are listed on the mixer record sheets along with use levels of all ingredients. Any tag number can be traced to a given production run. For drug runs, the serial numbers also identify the run on the daily production and control sheet. The mill calculated drug level is listed as well as the laboratory analysis if the run is sampled.

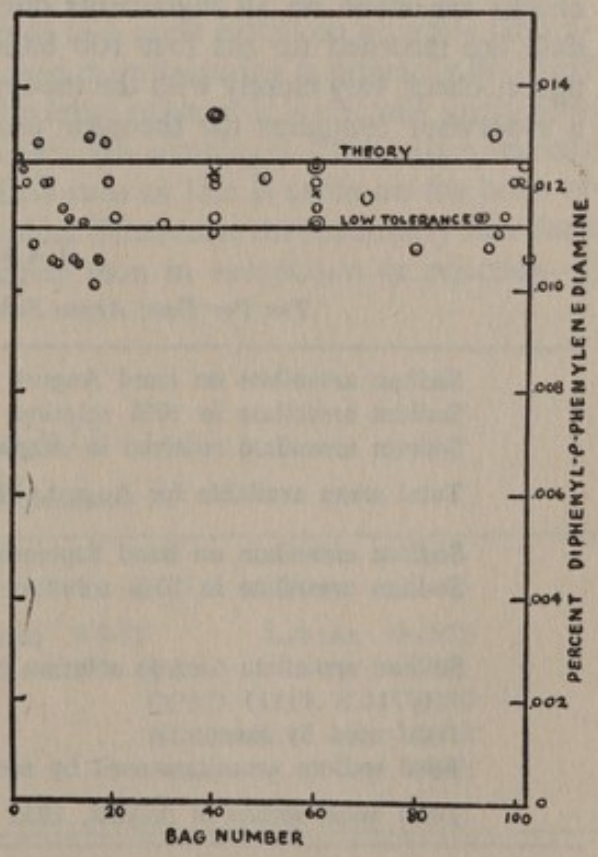

FIG. 3. Diphenyl-*p*-phenylenediamine distribution in run no. 4.

TABLE IV

Ten Per Cent Arsan Solution Use Record

Date used August 22, 1955	
Scale	Feed Improved Hi-Ener-G Nicarb Arsan
Start 537	Tag serial T 542109–T 544761
Stop 313	Cwts. made 2603
Used 224	Mill result .0096%

Laboratory analysis: no. 1, .0101%; no. 2, not run

Table II is the monthly inventory proof for August, 1955, covering the August 22 date on which the run was made. Generally speaking, the discrepancy between use and inventory values is no greater than 3 lb. per month for any drug at all plants.

Table III is a record of 10 per cent sodium arsanilate solution preparation. The actual concentration of the solution is 11.11 per cent. Batch-weight packages of drugs are used. The solution tanks are mounted on scales for accurate water additions. Table IV gives the use record for 10 per cent arsan solution for the run and again indicates close agreement between the mill calculated result and the laboratory analysis. Table V shows the monthly proof sheet for 10 per cent arsan solution for August, 1955. The inventory shrink was 2.27 lb. greater than indicated from mixer use records.

Table VI lists the drug use data for the 2603 bag run of the broiler ration containing both nicarbazin and sodium arsanilate. Accurate and frequent weight checks are made on all ingredients during the course of each run. For all runs, data are recorded for the first 100 bags and each succeeding 200 bags. The use figures check very closely with the theoretic figures. At the completion of each run, a supervisor compares the theoretic use with the actual use shown on the mixer

TABLE V

Ten Per Cent Arsan Solution Monthly Proof Sheet

Sodium arsanilate on hand August 1, 1955	1640	
Sodium arsanilate in 10% solution (229 × .1111)	25.44	
Sodium arsanilate received in August	None	
Total arsan available for August, 1955	1665.44	1665.44
Sodium arsanilate on hand September 1	660	
Sodium arsanilate in 10% solution	34.77	
	694.77	694.77
Sodium arsanilate used in solution by sheets (8716 × .1111)	968.40	
Total used by inventory		970.67
Total sodium arsanilate used by records, August		968.40
Total arsan minus in August, 1955		2.27 lb.

TABLE VI

Mixer Record Sheet for
Improved Hi-Ener-G Nicarb Arsan T 542109–T 544761

Bags	Nicarbazin used, lb.	10% Arsan solution used, lb.
0–100	5½	8½
101–300	10½	17
301–500	10	17½
501–700	10½	17½
701–900	10½	17½
901–1100	10½	17
1101–1300	10½	17½
1301–1500	10	17
1501–1700	10½	17
1701–1900	10½	17½
1901–2100	10½	17
2101–2300	10½	17½
2301–2500	10½	17
2501–2603	5½	8½
Total	136	224
Should use	136½	227½
Counter—2603	Start—1:56	End—4:06

records. Table VII shows a laboratory report on the 2603 bag run covered in the preceding tables. The analytic results on nicarbazin show a lab value of .0126 per cent versus a mill result of .0130 per cent and on sodium arsanilate, .0101 per cent versus .0096 per cent.

Successful use of feed medication depends to a great extent on accurate laboratory controls. Chemical control in modern feed manufacturing is primarily designed to prevent future errors from occurring. With large volumes it is virtually impossible to hold feed on the floor until laboratory assays are completed. Plant mixer records are accurate. Acceptance or rejection of feed runs or lots is made on the basis of these records with later laboratory confirmation. Therefore, the laboratory functions more in tightening operations and in policing than in acceptance or rejection of feed runs.

TABLE VII

Laboratory Analysis

Date made August 22, 1955
Tag serial T 542109–T 544761–1
Date received 8-29-55 Reported 9-9-55 Lab no. 0-4975

Protein	21.00	Salt	.63
Fiber	3.80	DPPD	.0130
Ash	5.63	Nicarbazin	.0126
Calcium	1.04	Arsan	.0101
Phosphorus	.71		

Development of rapid yet accurate chemical assays should be stressed. For example, 72 sulfaquinoxaline or sodium arsanilate determinations can be run daily by two technicians with good precision. Most other drugs require more time, which means the costs of control work are increased or a smaller number of samples can be run. Occasionally, two drugs are used in combination, and interference in the chemical determination occurs. The interference between sulfaquinoxaline and arsanilic acid in the determination of either drug is a good example. It is important that any tentative methods be rapidly circulated between the industry and feed control officials for collaborative study.

SUMMARY

1. Toxicity studies on most commercial feed-consuming animals should be made available.

2. If used as a dry premix, the compound should have granular, nonsegregating, nonelectrostatic properties for proper dispersion in manufactured feed.

3. Drug feeder location on the mixing line is important, with a locus nearest the mixer preferable.

4. If the drug is soluble, application through a liquid medium should be considered.

5. The disposition of the beginning and end of a run is a serious problem, which can be solved by proper application of chemical work. In this respect, results will differ between feed plants.

6. An accurate method of plant inventory and use control will contribute greatly to proper addition of drugs.

7. Rapid and precise laboratory methods are a most important part of an effective quality control program.

8. Because of feed manufacturing volume and rapid plant out-turn, laboratory analyses are used to a greater extent for the improvement of future plant procedures than as a basis of acceptance or rejection of plant runs. For this purpose, accurate mixer record data, followed by later confirmation in the laboratory, are indispensable.

9. Each new additive presents its own peculiar problems to the feed manufacturer. In handling these problems, basic improvements in the addition of other nutrients are also made. As new and better drugs are developed, problems for the feed manufacturer will increase. These are a constant challenge. With the cooperation of the Government, drug, and feed industry personnel, we can effectively meet this challenge.

The Feeding of High Levels of Diethylstilbestrol to Beef Steers

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Following the work of Burroughs et al,[1] the feeding of 10 mg. of diethylstilbestrol per day to fattening beef steers has become widely accepted. During the early use of diethylstilbestrol in commercial feed supplements, many questions were raised. What will happen if steers receive more than 10 mg./day? Does the feeding of diethylstilbestrol affect carcass quality? Does the feeding of diethylstilbestrol produce side effects, such as riding, fighting, bellowing, teat elongation, and high tail heads?

With these questions in mind and a desire to learn more about the metabolism of diethylstilbestrol in steers, an experiment was undertaken in which various levels of diethylstilbestrol were fed. It was hoped that the feeding of high levels would intensify any side effects that might be associated with administration of the substance.

PROCEDURE

Twenty-three Hereford steers, calved in one local herd, and one Shorthorn were divided into six groups of nearly equal weight (825 lb. ± 5 lb.). They were full fed by hand two times daily on ground corn and cob meal (80 per cent ground shelled corn and 20 per cent cob) plus 5 lb. of good quality alfalfa hay and 2 lb. of soybean oil meal per day. Iodized salt and a commercial mineral mix containing trace minerals were fed free choice.

The diethylstilbestrol was mixed into soybean oil meal at levels to give 10, 30, 50, 100, and 200 mg. in 2 lb. of meal per day. The bean meal was then mixed with the corn and cob meal.

The largest steer in each group was slaughtered on the twenty-eighth day of the test, within 12 hours of the last feeding of diethylstilbestrol (four hours after removal from the diethylstilbestrol supplement feed lot).

The second largest steer in each group was slaughtered on the sixty-second day of the test, 24 hours after the last feeding of diethylstilbestrol (16 hours after removal from the diethylstilbestrol supplement feed lot).

The remaining 2 steers in each group were slaughtered on the one hundred and fourth day of the experiment, 48 hours after their last feeding of diethylstilbestrol (40 hours after removal from the diethystilbestrol supplement feed lot).

The carcass weight was checked after 48 hours in the cooler and the per cent of cooler shrink was determined. The carcasses were ribbed down on the rail and

TABLE I

*Results of the Mouse Assay on Tissue and Fat from Steers Slaughtered 48 Hours after the Last Feeding of Diethylstilbestrol**

Tissue	Diethylstilbestrol, μg./Gm. (dry wt. basis), in steers fed				
	10 mg.	30 mg.	50 mg.	100 mg.	200 mg.
Lean meat	0.0	0.0	0.0	0.0	0.0
Fat	0.0	0.0	0.0	0.0	0.0
Liver	0.0	0.0	0.0	0.0152	0.02216
Kidney	0.0	0.0	0.0	0.01372	0.01552
Tripe	0.0	0.0	0.0	0.0368	0.432

* Figures in excess of .003 μg. are significant.

graded. Samples of lean meat were checked for fat, water, and nitrogen content. Samples of kidney fat were checked for water content. Samples of liver, kidney, tripe, lean meat, and fat were taken for assay of estrogenic activity. A modification of the estrogen assay as described by Preston et al[2] was used in this experiment.

All tissues for the estrogen assay were ground, frozen, and vacuum dried. The fat was rendered by heating at 100 C. for 30 minutes and recovering the liquid portion. All materials were stored at 5 C. until diets were prepared.

A regular small-animal laboratory ration was used as the basal mouse diet and the dry tissues were added in the following concentrations: lean meat, 70 per cent; liver, 50 per cent; kidney, 20 to 40 per cent; tripe, 50 per cent; fat, 20 per cent.

Three diets were prepared from each tissue for the assay. To obtain optimum sensitivity and directly comparable results, .005, .01, and .02 μg. of diethystilbestrol were added per Gm. of diet.

Immature female white mice weighing 10 to 12 Gm. were divided into groups of 20 and fed the experimental diets two times daily for seven days. On the eighth day, the uteri were removed, fixed in Bouin's solution, trimmed, and weighed.

RESULTS

The data are recorded in tables I to IV. The weight gains of the control, 10, 30, 50 and 100 mg. groups were not significantly different. The 200 mg. group gained considerably less, which is correlated with their depressed appetites during the experiment.

TABLE II

*Results of the Mouse Assay on Tissue and Fat from Steer Slaughtered 24 Hours after the Last Feeding of Diethylstilbestrol**

Tissue	Diethylstilbestrol, μg./Gm. (dry wt. basis), in steers fed				
	10 mg.	30 mg.	50 mg.	100 mg.	200 mg.
Lean meat	0.0	0.0	0.0	0.0	0.0
Fat	0.0	0.0	0.0	0.0	0.0
Kidney	0.0	0.005+	0.005+	0.005+	0.005+
Tripe	0.0	0.034+	0.034+	0.034+	0.034+
Liver	0.0059	0.070	0.1482	0.20+	0.20+

* Figures in excess of .003 μg. are significant.

TABLE III

*Results of the Mouse Assay on Tissue and Fat from Steers Slaughtered 12 Hours after the Last Feeding of Diethylstilbestrol**

Tissue	Diethylstilbestrol, μg./Gm. (dry wt. basis), in steers fed				
	10 mg.	30 mg.	50 mg.	100 mg.	200 mg.
Lean meat	0.0	0.0	0.0	0.0	0.0
Fat	0.0	0.0	0.0	0.0	0.0
Bleached tripe	0.0	0.0	0.0	0.0048	0.096
Washed tripe	0.042	0.046	0.04+	0.04+	0.04+
Kidney	0.0066	0.03	0.04+	0.04+	0.04+
Liver	0.0174	0.0632	0.20+	0.20+	0.20+

* Figures in excess of .003 μg. are significant.

Table I represents the results of the mouse assay on tissue and fat from steers slaughtered 48 hours after the last feeding of diethylstilbestrol. No residue was found in any of the tissues in the 10, 30, and 50 mg. fed steers. Residues were found in the tripe, liver, and kidneys of the 100 and 200 mg. fed steers.

Table II represents the results of the mouse assay on tissue and fat from steers slaughtered 24 hours after the last feeding of diethylstilbestrol. A small amount of residue was detectable in the liver of the 10 mg. fed steer at this time. Based on the amount detected here, one would have to consume one third of a pound of liver three times a day for 1260 days to obtain an equivalent activity comparable to a 1 mg. dose.

Table III represents the results of the mouse assay on tissue and fat from steers slaughtered 12 hours after the last feeding of diethylstilbestrol. No residue was found in the lean meat or fat of any of these animals, regardless of the amount of diethylstilbestrol being fed. Residue was found here in tripe, liver, and kidney of the 10 mg. fed steer.

Table IV represents the carcass grade, cooler shrinkage, and laboratory analy-

TABLE IV

Average of Data on All Carcasses

Feeding levels	Chill shrink, %	Carcass grade	Nitrogen, %	Water, %	Fat, %	Water in fat, %*
Control	3.0	2 Choice 2 Good	3.30	72.50	22.47	4.49
10 mg.	3.0	3 Choice 1 L. choice	3.40	73.54	22.35	3.45
30 mg.	3.2	4 Choice	3.31	73.75	18.79	5.64
50 mg.	3.2	3 Choice 1 Good	3.39	74.48	13.75	5.28
100 mg.	2.9	3 Choice 1 Good	3.41	73.80	14.65	4.32
200 mg.	2.8	3 Choice 1 Good	3.41	73.84	17.84	5.94

* The water content of the fat on the first 6 steers slaughtered was not determined.

sis obtained on each group of animals in this experiment. The differences here do not appear significant nor do they indicate a trend that can be correlated with increasing dosages of diethylstilbestrol.

CONCLUSIONS

1. No estrogenic residues were detected in the lean meat or fat of cattle fed as much as 200 mg. of diethylstilbestrol, regardless of the time interval between the last feeding of diethylstilbestrol and slaughter.

2. Trace amounts of residue can be detected in the liver and kidney while the gut is filled with diethylstilbestrol-containing feed, but these traces disappear gradually with the normal passage of the fluid ingesta between 24 and 48 hours after the last consumption.

3. The feeding of 10, 30, and 50 mg. of diethylstilbestrol per day to 825 pound beef steers for as long as 104 days produced no unfavorable side effects.

4. Animals that were fed 100 and 200 mg. per day developed a sagging loin area (high tail head).

5. The appetites were depressed and weight gains reduced when 200 mg. per day was fed.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of the Eli Lilly & Co. technicians in the completion of this project.

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The Control of Coccidiosis and Enterohepatitis with Medicated Feeds

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It is possible, with medicated feeds, to achieve a degree of control not possible with any other practical methods against two of the most costly infectious diseases of poultry in the United States, namely, coccidiosis in chickens and enterohepatitis (blackhead disease) in turkeys. These are both caused by protozoan parasites, the first, by several species of the coccidial genus *Eimeria* and the second, by the flagellated protozoan *Histomonas meleagridis*. It is estimated by the United States Department of Agriculture that if coccidiosis were uncontrolled today, it would cost the poultry industry of the United States about 112 million dollars annually at the present level of production. Between the years 1890 and 1920, there was a drop in turkey production from 11 million to 3.5 million birds annually, in spite of a marked increase in the population of the United States during that period. This was most likely due to blackhead disease, which made turkey raising on a commercial basis almost impossible. The first step in the control of this scourge of the turkey flocks was the discovery of the method of transmission and the recognition of the importance of chickens as carriers by Tyzzer at Harvard University. Separation of chickens from turkeys and the rearing of turkeys on wire-floored platforms or with careful range rotation brought this disease under reasonable control and allowed the industry to develop extensively. These methods of rearing were costly, and blackhead disease still remained as a constant threat to the turkey raiser until the historical discovery of the drug 2-amino-5-nitrothiazole* by Waletzky[14] and its wide adoption by the industry. For the most part, this disease is no longer terrifying to turkey raisers, since they know it can either be prevented or controlled by the proper use of 2-acetylamino-5-nitrothiazole in the feed or drinking water. Certain other drugs may be useful in prevention, as will be discussed later.

Sanitary or management measures alone have never been particularly successful in reducing losses from coccidiosis, chiefly because they are incompatible with the crowding and economy that is necessary in commercial operations for the profitable mass production of poultry. Only the continuous use of some of the recently developed anticoccidials in the feed for prevention has solved the coccidiosis problem economically and feasibly. It is estimated that the present use of

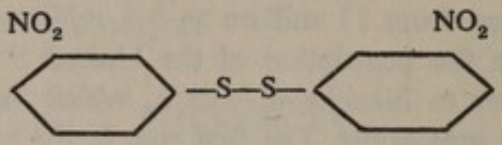
* The trade name of American Cyanamid Co. for 2-amino-5-nitrothiazole is Enheptin. This has now been replaced by the more active derivative, 2-acetylamino-5-nitrothiazole. The trade name of the American Cyanamid Co. for the latter compound is Enheptin-A.

these drugs for the prevention of coccidiosis has reduced the potential annual losses of 112 million dollars down to less than one fifth of this figure, and possibly less than one tenth.

COCCIDIOSIS

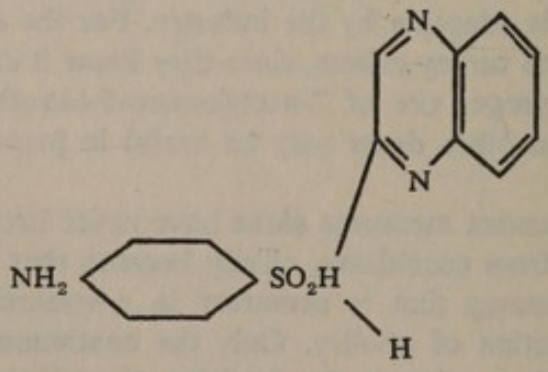
The first drug shown to have any marked effect against the coccidia of chickens was sulfanilamide.[8] This quickly led to the finding that sulfaguanidine was more active, and this drug was soon used widely to stop outbreaks of the disease. Waletzky and Hughes[12] made an extensive study of large numbers of sulfonamides and found that their anticoccidial activity, like their antibacterial activity, was closely related to the blood levels attained following the feeding of the drugs. From these studies, sulfamethazine* was chosen as the most active and economical of the group, and this drug in drinking water or feed came to be the accepted method of treating outbreaks of coccidiosis.

Parasitologists and poultry scientists argued that it might be more effective to prevent outbreaks of coccidiosis by feeding a suitable drug continuously rather than waiting until an outbreak occurred to treat it. The search for drugs of sufficiently high activity and low cost to permit continuous feeding was pursued most actively in a number of industrial laboratories, chiefly because of the availability of many different chemical compounds. In our own laboratories many thousands of widely differing compounds were screened in this search which eventually led to the selection of nitrophenide.†



m,m'-Dinitrodiphenyl disulfide
(nitrophenide)

Other notable developments came from the laboratories of Merck and Co. where they also followed the sulfonamide lead and came forth with sulfaquinoxaline whose activity in continuous feeding was reported by Grumbles et al[17] at the University of Rhode Island.

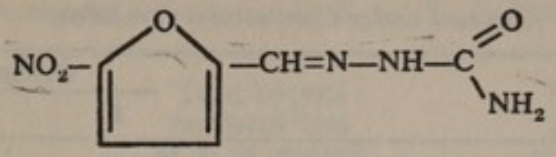


Sulfaquinoxaline

* The trade name of American Cyanamid Co. for sulfamethazine is Sulmet.

† The trade mark of American Cyanamid Co. for a 25 per cent premix of nitrophenide is Megasul.

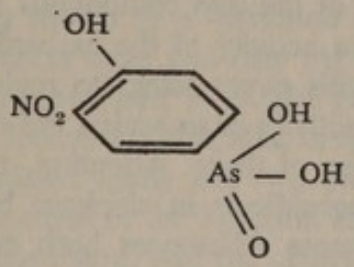
Harwood and Stuntz[6] tested a series of furfural derivatives and selected nitrofurazone as the most promising for development as a preventive drug for coccidiosis.



5-Nitro-2 furaldehyde semicarbazone
(nitrofurazone)

These three drugs, nitrophenide, sulfaquinoxaline, and nitrofurazone, all introduced at about the same time (1949), launched a new approach to coccidiosis and became the standard drugs for coccidiosis prevention.

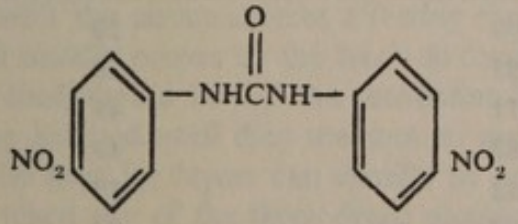
Two other compounds or groups should be mentioned. The chemical 3 nitro-4-hydroxy phenyl arsonic acid was selected as a result of the studies on various arsenicals by Morehouse and Mayfield.[11]



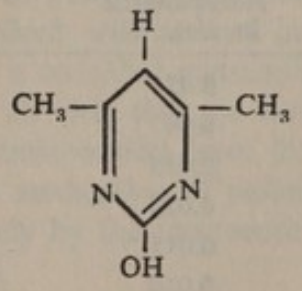
3-Nitro-4-hydroxy phenyl
arsonic acid

This arsenical has some activity against the causative agent of cecal coccidiosis, *Eimeria tenella,* but, unlike the three compounds mentioned, it evidently strikes the parasites only when they are initially released from the oocysts in the lumen of the intestine, since it is ineffective after the parasites have entered the epithelial cells of the intestinal mucosa. Also, it has questionable or no activity against other species of coccidia.

Recently, Merck & Co. introduced a new type of compound, or actually a complex, that is highly active against at least three of the species of coccidia, *E. tenella, E. necatrix,* and *E. acervulina,* found in chickens.[4] This drug, called nicarbazin, is a complex of two chemicals, 4,4'-dinitrocarbanilide and 2 hydroxy-4,6-dimethylpyrimidine.



4,4'-Dinitrocarbanilide



2 Hydroxy-4,6-
dimethylpyrimidine

TABLE I

*The Effect of Nitrophenide, When Fed Continuously from Hatching to Market, on the Mortality, Weight Gains, and Feed Conversion of Broilers Raised under Commercial Conditions**

	Group number		
	1	2	3
Number of birds	1800	900	900
Nitrophenide in diet	none	0.0125%	0.025%
Deaths from cecal coccidiosis, per cent	3.00	0.22	0.11
Deaths from intestinal coccidiosis, per cent	1.30		0.11
Average weight of birds when sold, lb.	3.65	3.82	3.98
Lb. of feed consumed/lb. of body weight gain	3.64	3.45	3.30

* Unpublished data.[3]

This drug is particularly interesting scientifically because of the synergistic effect of the complex. The second of the two compounds alone has little or no activity, but its presence increases the activity of the carbanilide by about tenfold.

No attempt is made in this paper either to review all of the compounds that have shown some degree of activity or to review the different commercial variations that have been made of some of these. Attention, rather, is given to the general principles of dealing with coccidiosis in chickens by means of medicated feeds. While there are obviously some differences both quantitatively and qualitatively between the various drugs, the general comments that follow can be taken to apply to nitrophenide, sulfaquinoxaline, nitrofurazone, and nicarbazin. The arsenicals are not included in this list because of their restricted activity and because of the complicating factor of their supposed growth-stimulating effect, which is not related to their anticoccidial activity.

Following the laboratory demonstration of the anticoccidial activity of these drugs, the field studies by many different groups brought out important points regarding the biology of coccidiosis and its prevention. Not only is an outbreak

TABLE II

The Effect of Graded Doses of Nitrophenide on the Survival of 10 Day Old Chicks Each Experimentally Infected with 500,000 Oocysts of E. tenella*

Nitrophenide in diet, %	% Birds alive at 8 days, av. of several groups	Total no. birds/treatment
0.05	100	50
0.04	95	20
0.035	71	48
0.025	82	49
0.015	52	29
0.010	74	27
none	40	99

* Adapted from Waletzky et al.[13]

TABLE III

The Effect of Graded Doses of Nitrophenide on the Production of Oocysts of Experimental Infections with E. tenella *in Chickens**

Nitrophenide in diet, %	Total oocysts produced/bird as % of controls	Viable oocysts produced/bird as % of controls
0.025	2.6	1
0.02	13	5
0.0125	55	22
0.01	72	29

* Adapted from Brackett and Bliznick.[2]

of clinical coccidiosis prevented by the continuous feeding of these drugs from the time the chicks are hatched, as shown by a reduction in mortality, but in addition the chickens weigh more at market time, and they eat less feed per pound of body weight gain (table I). This improvement in weight gains and feed efficiency is a regular occurrence when an effective anticoccidial drug is used.

It is now clear that coccidiosis really has two phases: the subclinical phase, which occurs during the development of the infection and may interfere with growth and which causes poor feed conversion, and the clinical stage, which is marked by mortality. In many, if not most, cases, the economic losses during the subclinical stages may be as great as or greater than those resulting from mortality. It was also learned that a smaller concentration of drug in the feed is required to prevent an outbreak than to treat an outbreak. The drug levels used for prevention, e.g., 0.0125 to 0.025 per cent nitrophenide, will not completely prevent mortality in an acute outbreak (table II), but these lower levels will cut down oocyst production (table III). Since the infections are transmitted and built up by means of these oocysts, any reduction in numbers will slow the build-up of an infection in a flock. The light infections that occur early do confer some resistance on the chickens, and it is now believed the mechanism of action of at least nitrophenide, sulfaquinoxaline, and nitrofurazone is to slow the build-up of infections enough so that immunity develops before the litter reaches dangerous levels of contamination with oocysts. When the chickens become immune to a species of coccidia, they are safe from this species. When they become immune to all dangerous species, they no longer need drug protection. The time at which a flock will become immune varies with the circumstances affecting the build-up of a coccidial contamination, but this usually occurs by the tenth to fourteenth week. Broilers that are marketed at this time should be fed the preventive levels of an anticoccidial from the time they are hatched until they are sent to market, but the medication of pullets that are to be kept for layers can usually be terminated safely by the fourteenth week of age when any of the three drugs mentioned are used.

Nicarbazin may prove to be somewhat different in its mechanism than these three drugs although, because of its newness, the technical story is incomplete as yet. In their literature the manufacturers indicate a complete or almost complete

TABLE IV

The Effect of Graded Doses of Nicarbazin on the Production of Oocysts of Mixed Experimental Infections with E. tenella *and* E. necatrix*

Nicarbazin in diet of chickens, %	Millions of oocysts/ chick†
none	28
0.005	31
0.01	1
0.02	<0.1
0.04	<0.1

* Data taken from Merck technical bulletin on nicarbazin[10].
† Average of 20 chicks per group.

elimination of oocyst production when recommended levels of nicarbazin are used (table IV). This could mean complete prevention of coccidiosis with nicarbazin in contrast to the limited infections permitted by the other three drugs. In the absence of infection it is likely that immunity may not develop during the period of nicarbazin feeding. In broilers, which can be medicated throughout the entire 8 to 12 week growing period, the lack of immunity would be no disadvantage, and the complete prevention of all infection would be an advantage. With replacement birds, the situation is not the same. If for economy's sake medication will be terminated at some time during the life of the birds, or if, because the drug cannot be fed safely on into the laying period, as is the case with nicarbazin, then it is vital that the birds possess some resistance to coccidiosis when they no longer enjoy the protection conferred by a drug.

The cost of preventive medication for coccidiosis for the first 10 to 12 weeks of life is about 1 cent/bird or 10 dollars/thousand. The savings in weight gains, birds, and feed is usually worth an average of at least 5 cents/bird or 50 dollars/thousand, or a return of 5 dollars for every dollar invested. This is invariably far better than waiting to treat an outbreak, since by the time coccidiosis is in evidence, the flock has already suffered the greatest proportion of potential coccidiosis losses. At best, treating an outbreak will save only a few birds from dying, while preventive medication will not only save these birds but will also prevent weight and feed losses as well. In table V, it can be seen that group 3 on therapeutic medication was no better than the untreated group, while group 2, which was fed anticoccidials continuously for preventive purposes, was better than either the untreated or the therapeutically treated groups. It is for these reasons that the author strongly feels that anticoccidial medication would be advisable as a standard practice in all feeds for chickens during the first 10 to 14 weeks of life.

ENTEROHEPATITIS

There are now at least three drugs used in the treatment or prevention of blackhead disease of turkeys. The first of these to be used was 2-amino-5-

TABLE V

*A Summary of Experimental Data Comparing the Results of Treating Outbreaks of Coccidiosis with Prevention by Continuous Feeding in Broilers Raised under Commercial Conditions**

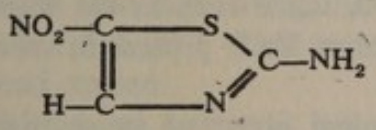
	Method of medication	No. birds	Av. market wt., lb.	Lb. feed/lb. of gain	Mortality		
					Total, %	Cecal coccidiosis, %	Intestinal coccidiosis, %
1	None	2400	3.15	2.91	6.8	0.2	1.4
2	Continuous feeding of anticoccidials from hatching to market†	7200	3.34	2.86	3.6	0.1	—
3	Spot treatment of outbreaks when they occurred‡	7200	3.05	2.93	7.5	0.3	1.1

* Unpublished data.[16]

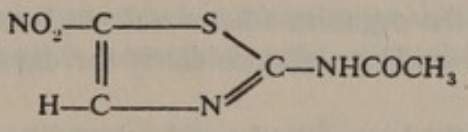
† Nitrophenide, nitrofurazone, and sulfaquinoxaline fed to different groups according to standard recommendations of the manufacturer of each.

‡ Therapeutic treatment with sulfamethazine and sulfaquinoxaline under the direction of experienced personnel and according to the manufacturer's directions for use.

nitrothiazole, which has already been mentioned. This drug was discovered by testing, in experimentally produced enterohepatitis in turkeys, chemicals that had shown definite but low levels of activity against coccidiosis. Of 18 types tested by Waletzky,[14] 2-amino-5-nitrothiazole was found to be highly active both in treating advanced infections as well as preventing these infections when medication was started before inoculation or exposure. This drug and its more active analogue, 2-acetylamino-5-nitrothiazole, act in essentially the same fashion. Subsequent remarks can be taken to refer in general to either one, except for quantitative differences, but 2-acetylamino-5-nitrothiazole will be discussed.



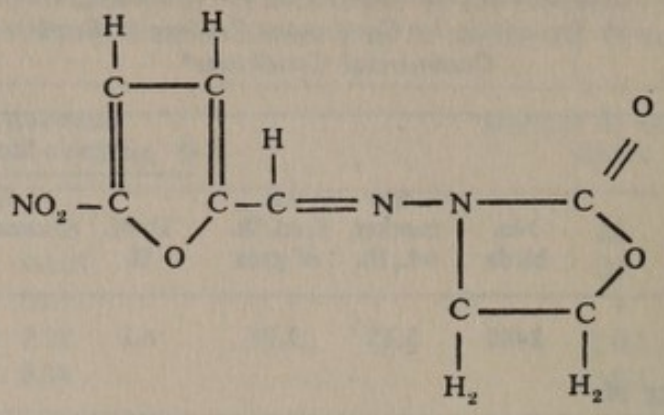
2-Amino-5-nitrothiazole



2-Acetylamino-5-nitrothiazole

An arsenical, 4-nitrophenylarsonic acid, was shown by McGuire and Morehouse[9] to be active in preventing infections if medication is started before and continued during periods of exposure, but it will not stop an infection once started in an individual turkey. This drug has evidently been used widely under practical conditions with success.

Most recently claims have been made for furazolidone as a preventive for enterohepatitis, although the available data is very sparse.[5, 7]



Furazolidone

Blackhead disease differs markedly from coccidiosis in a number of basic biologic respects, which have an important bearing on the methods used to control this disease. The most important differences are as follows.

Blackhead disease is qualitative, while coccidiosis is quantitative. By this it is meant that any degree of exposure of turkeys to the causative agent of enterohepatitis will lead to a full clinical infection with mortality unless checked by an effective drug. In coccidiosis, exposure to a few oocysts results in only mild infections, while large numbers of oocysts are required to give severe infections, and even then death may not be inevitable.

Immunity follows quickly and powerfully in chickens exposed to the oocysts of coccidia, while none or only extremely limited immunity results from the infections with the agent of enterohepatitis in turkeys.

Coccidiosis is probably present to some degree in every flock of chickens, especially if they are reared on the floor or ground rather than in wire-floored cages. It can be present without any obvious evidence, so the grower may be unaware of it, yet suffer financial losses unless effective medication is used. However, if conditions are right, the infections may develop to clinical proportions. The organism causing enterohepatitis is widely distributed but will not exist in a flock unnoticed because of its unlimited ability to multiply in an infected turkey until sickness and death ensues. Thus, the absence of clinical blackhead in a flock means the organisms have not yet been introduced, while even in the absence of clinical evidence of coccidiosis the disease is still most likely present in subclinical levels.

Clinical coccidiosis with mortality is the terminal phase of an outbreak. At this time immunity develops, the flock recovers, and it needs no further protection. Clinical cases of blackhead, on the other hand, are first seen in the early phases of the infection in a flock and may be followed by many more cases over a long period of time.

Because of the severity of the cecal and liver damage resulting from blackhead infections, the high mortality of natural and experimental infections, and the lack of immunity, it was felt that the only kind of drug that would be useful would be one that completely prevented the infections. One of the remarkable features that

TABLE VI

The Effect of Medication with Enheptin and Enheptin-A on Turkeys When Started at Different Times after Inoculation with Histomonas-*infected Cecal Worm Eggs and Continued for 14 days**

Day after inoculation on which medication was started	% Turkeys alive†		
	No drug	0.05% Enheptin-A in feed	0.05% Enheptin in feed
4	0	45	44
7	0	52	71
10	0	63	61
11	3–8	83	75
12	0	72	94
14	1–2	72‡	44‡
Total no. birds in all trials	172	174	121

* Unpublished data.[15]

† The mortality figures include deaths during medication and following medication through the relapse period.

‡ These figures do not include deaths that occurred before or on the first day of medication.

was discovered about 2-amino-5-nitrothiazole and 2-acetylamino-5-nitrothiazole was their ability to save infected birds even when the drug treatment was started only a few days before the birds would otherwise have died from the infection (table VI). 2-Acetylamino-5-nitrothiazole may also be used as a preventive, though, by feeding it continuously during the periods when exposure to the infection is common.

Because of the biologic characteristics of blackhead disease and the chemotherapeutic properties of 2-acetylamino-5-nitrothiazole and other drugs, the following practical principles in the control of this disease have evolved from experimentation and experience.

Prevention is feasible and desirable whenever the disease occurs regularly and extensively. Medication should be started a few weeks prior to the period which experience indicates is likely to be the beginning of the disease season in each specific area. For this purpose, the preventive level of the drug is fed continuously in the diet, calculated on a complete feed basis. If the ration consists of half formulated feed and half grain, then the recommended level must be doubled in the formulated feed. The preventive feeding is continued until after the usual blackhead season.

If blackhead disease is not of regular occurrence, it is possible to wait until the first signs of disease in a flock and then to administer 2-acetylamino-5-nitrothiazole at the therapeutic level of 0.05 per cent drug on a complete feed basis until the outbreak is brought under control, usually not over a week or 10 days. Following that, two alternatives are available and the selection depends on experience and personal desires. The first is: terminate medication with 2-acetylamino-5-nitrothiazole and repeat only if and when a relapse occurs. This constitutes something of a chance, since it is known that the premises are now infected and that turkeys do not become immune to the disease. Therefore, the

following alternative is probably the desirable one: switch to the preventive level of drug and continue this feeding until the blackhead season is over.

2-Amino-5-nitrothiazole can be used in the drinking water, but this is usually not a convenient method of administration for long periods of time. Therefore, it is used only for treating an outbreak or supplementing the preventive level in the feed in those rare instances when exposure is so heavy that it overwhelms this level and some clinical enterohepatitis occurs in spite of the preventive feeding. This latter use simplifies the feeding program by making it necessary to have only one type of feed, i.e., only the preventive level rather than both it and the therapeutic level.

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Labeling of Medicated Feeds from the Feed Manufacturers Point of View

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After approximately a decade of medicated feed manufacturing, we of the feed industry believe it is in order to review constructively our present method of labeling medicated feeds. In reviewing the label our prime consideration should be for the feeder. The information on our tags must guide him in proper usage of the medicated feed. We have expressed our opinions on the present methods of labeling at several recent meetings with control officials. In spite of some differences of opinion, we believe our aim is common: that of providing the feeder or purchaser with a feed tag that will tell him what he is buying, how he should feed it, and what are its safe limitations.

On the foregoing basis, we shall discuss the two questions relative to present medicated labeling that we in the industry think merits consideration. Can our present method of labeling be simplified for the benefit of the feeder or purchaser? Should we afford the trade or brand names more prominence on the label?

We believe when drugs or medications are added to the label statement, this addition should be expressed in language and copy likely to be read and understood by the average feeder. In our opinion it is questionable whether the chemical names, such as oxytetracycline, chlortetracycline, 2-acetylamino-5-nitrothiazole, or furazolidone, although they be the common English generic names of antibiotics and drugs currently used, belong as part of the medicated feed name or make the tag more likely to be read and, therefore, be understood. Consider also that most of these drugs are marketed and advertised under trade names. These trade names are the names familiar to the feeder and in some cases are not associated with the common English generic names found on current labeling. Because of these long unfamiliar terms, I do not know of any feeder or dealer who orders or discusses these medications in cleared nomenclature, for example, nitrophenide mixture incorporated in Orion Broiler Mash.

Our experience has been that shortened names using suffixed symbols facilitates accurate ordering, manufacturing, and distribution of medicated feed in the trade. This we believe to be true of other manufacturers. Take a look at our present type of tag (table I). This is a typical medicated-feed tag now being widely used having three commonly used medications: an antibiotic, a coccidiostat, and a growth stimulant. We would be remiss if, in differing with the present label, we did not suggest one we consider more desirable from the feeder's standpoint.

Consider table II. This follows some of the thinking and discussion of the May, 1955, meeting of the Medicated Feed Labeling Committee in joint sessions

TABLE I

Medicated Feed Tag Presently in Use

ORION
CHLORTETRACYCLINE
(AUREOMYCIN)
NICARBAZIN AND 3-NITRO MIXTURE

ACTIVE DRUG INGREDIENT:

Chlortetracycline equivalent to Chlortetracycline Hydrochloride 0.05 grams per pound (100 grams per ton).

Nicarbazin	0.0125%
3-Nitro 4-Hydroxphenylarsonic Acid	.005%

For stimulation of feed intake and maintenance of weight gains in chicken flocks in the presence of chronic respiratory disease (air sac disease) and a preventive against outbreaks of Cecal and Intestinal Coccidiosis and for stimulating growth in chicken flocks.

INCORPORATED IN ORION BROILER MASH

GUARANTEED ANALYSIS

Crude Protein, not less than	22.00%
Crude Fat, not less than	4.00%
Crude Fibre, not MORE than	4.00%
Nitrogen-free Extract, not less than	49.00%

INGREDIENTS: Ground Corn, Corn Gluten Meal, Ground Oats, Meat and Bone Scraps, Dehydrated Alfalfa Meal, Soybean Oil Meal, Fish Meal, dl Methionine, Di-Calcium Phosphate .5%, Animal Fat (preserved with diphenyl paraphenylenediamine) Vitamin A and D Feeding Oil, D Activated Animal Sterol, Calcium Pantothenate, Vitamin B-12 Supplement, Antibiotic Feed Supplement, Niacin, Ground Limestone 1%, Salt .5%, Manganese Sulphate .03%, Potassium Iodide .0025%.

ORION FEED MILLS, SALISBURY, MARYLAND
Important to follow directions on back of tag.

of control and industry members. This tag is, we think, simple, direct, and understandable. The word "medicated" directly following the brand name alerts the feeder to the fact that this broiler mash contains a drug or drugs. The suffixed symbol A3Z defines this as a specific medicated broiler mash. Other broiler mashes medicated by the same manufacturers could have other symbols appropriately suffixed according to drugs added. The active drug content directly following the brand name defines the drugs added and their concentration in their common generic names. These should be printed in bold type to make them stand out. The definition of purpose is specific for the feeding as well as the drugs contained. Trade and brand names are given proper emphasis. Other required information on the tag is present.

This type of labeling is currently being used on other products having active ingredients. These labels are presumably informative and adequate from the purchaser's point of view. The word "medicated" seems to be a well-understood term, which flags the purchaser's attention and invites his interest to further read what the active ingredients are.

We feel that trade-marks and/or brand names should not be obscured by a list of long chemical names as part of the brand name unless they are handled in the manner of table III. Some states now accept such labeling. We feel strongly that

the trade marks and brand names deserve a prominent place on any tag or label. They are informative, they are the basis of sound judgment in the purchase of feed.

We have in our industry trade-marks and brand names which, because of the work, research, services afforded, and actual performance of these trade-marked feeds in the feeding of poultry and animals, carry the significance of consistent quality. We would point out further that most of the feeds now being used as a vehicle for administration of these medicated additives existed for many years. Many feed purchasers were long familiar with the cost of results obtained on these feeds before the era of medicated additives. The purchaser of these products still thinks of these medicated feeds as feeds first, containing the desired medications for his specific need.

These thoughts are offered for constructive consideration. In the final analysis I am sure the responsibility for clear labeling lies with industry. It is we who will be taken to task if that label does not tell the whole story and a feeder gets into difficulty. We do not wish to infer from this paper that labeling of these products has been poorly conceived. We are cognizant of the effort that the Federal and state control officials have put forth to guide us in this era of medication.

TABLE II

A Proposal to Simplify Labeling of Medicated Feeds

ORION
BROILER MASH MEDICATED
A3Z

ACTIVE DRUG INGREDIENTS:

CHLORTETRACYCLINE from Aureomycin equivalent to Chlotetracycline Hydrochloride 0.05 grams per pound (100 grams per ton).

Nicarbazin	0.0125%
3-Nitro 4-Hydroxyphenylarsonic Acid	0.005%

A Broiler Mash containing medications for stimulation of feed intake and maintenance of weight gains in presence of chronic respiratory disease (air sac disease); for prevention against outbreaks of Cecal and Intestinal Coccidiosis; and for stimulation of growth in broiler (chicken) flocks.

INCORPORATED IN ORION BROILER MASH

GUARANTEED ANALYSIS

Crude Protein, not less than	22.00%
Crude Fat, not less than	4.00%
Crude Fibre, not MORE than	4.00%
Nitrogen-free Extract, not less than	49.00%

INGREDIENTS: Ground Corn, Corn Gluten Meal, Ground Oats, Meat and Bone Scraps, Dehydrated Alfalfa Meal, Soybean Oil Meal, Fish Meal, dl Methionine, Di-Calcium Phosphate .5%, Animal Fat (preserved with diphenyl paraphenylenediamine) Vitamin A and D Feeding Oil, D Activated Animal Sterol, Calcium Pantothenate, Vitamin B-12 Supplement, Antibiotic Feed Supplement, Niacin, Ground Limestone 1%, Salt .5%, Manganese Sulphate .03%, Potassium Iodide .0025%.

ORION FEED MILLS, SALISBURY, MARYLAND
Important to follow directions on back of tag.

TABLE III

An Alternative Proposal to Simplify Labeling of Medicated Feeds

ORION
BROILER MASH MEDICATED
CONTAINING CHLORTETRACYCLINE (from Aureomycin)
0.0125% Nicarbazin, and 3-Nitro.

ACTIVE DRUG INGREDIENT:

CHLORTETRACYCLINE from Aureomycin equivalent to Chlotetracycline Hydrochloride 0.05 grams per pound (100 grams per ton).

Nicarbazin	0.0125%
3-Nitro 4-Hydroxyphenylarsonic Acid	0.005%

A Broiler Mash containing medications for stimulation of feed intake and maintenance of weight gains in presence of chronic respiratory disease (air sac disease); for prevention against outbreaks of Cecal and Intestinal Coccidiosis; and for stimulation of growth in broiler (chicken) flocks.

INCORPORATED IN ORION BROILER MASH

GUARANTEED ANALYSIS

Crude Protein, not less than	22.00%
Crude Fat, not less than	4.00%
Crude Fibre, not MORE than	4.00%
Nitrogen-free Extract, not less than	49.00%

INGREDIENTS: Ground Corn, Corn Gluten Meal, Ground Oats, Meat and Bone Scraps, Dehydrated Alfalfa Meal, Soybean Oil Meal, Fish Meal, dl Methionine, Di-Calcium Phosphate .5%, Animal Fat (preserved with diphenyl paraphenylenediamine) Vitamin A and D Feeding Oil, D Activated Animal Sterol, Calcium Pantothenate, Vitamin B-12 Supplement, Antibiotic Feed Supplement, Niacin, Ground Limestone 1%, Salt .5%, Manganese Sulphate .03%, Potassium Iodide .0025%.

ORION FEED MILLS, SALISBURY, MARYLAND
Important to follow directions on back of tag.

The amount of uniformity that has been gained is, of course, important. We think this can be maintained. Our feed labeling is sure to change for the better as we of the industry work with you in control on that common aim of providing the feeder with the tag that tells him what he is buying, how he should feed it, and what are the safe limitations on these medicated feeds.

Tissue Residues of Drugs from the Use of Medicated Feeds

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Department of Health, Education, and Welfare
Washington, D. C.

The problem of residues, which occur in the edible tissues of animals as a result of drugs that the animals have received, is by no means a new one. It is, however, one that is assuming increasing proportions through a combination of circumstances. The most important of these is that the use of drugs is no longer limited to sick animals. As you all know, a number of substances have been found that, though they differ widely in their chemical nature and even in their mechanism of action, have the common property of accelerating the growth of farm animals and/or increasing their food efficiency. Such discoveries were important in themselves but their wide application rests on the additional feature that these drugs can be administered by incorporating them directly in the animal feed. There is a beautiful simplicity about this method of medication: no injections, no balling gun, no drenches, no calculation of dosage; simply shovel out the treated feed. Unless the hired hand reads the label on the feed sacks carefully, he may be scarcely aware that he is giving the animals medicine.

Granted then that the use of medicated feeds is assuming tremendous proportions, is there really a health problem from the possible tissue residues in the treated animals? An understandable reaction of those who first learn of the problem is "What in the world is all the fuss about? Surely, if the drug itself is safe for the animal, the minute amount that might find its way into the meat would not be enough to hurt anybody."

There are several facts that prevent us from reaching such a reassuring conclusion. The first of these is the amazing capacity of animals to store certain dietary ingredients in their tissues. A notable example of this is found in the case of the insecticide DDT. If this is added to the diet of rats in amounts of only 1 part per million, it will accumulate in the rat to a point where the fat contains a concentration some 30 times as much as was present in the diet. Similarly, when phenylmercuric acetate is fed to rats at about 1 part per million for a year, the level of mercury in the kidneys reaches a level 35 times that which was in the diet. As a final example, vitamin A can be stored in the liver of steers in sufficient amounts to make the livers toxic. The toxic effects of polar bear liver are similarly attributed to its high vitamin A content. From these examples, it is clear that the apparent well-being of an animal is no assurance that it has not stored dangerous amounts of some dietary ingredient in its tissues. Furthermore, depending on the nature of

the toxicant, it may be distributed rather generally throughout the body or selectively stored in one organ or tissue.

The situation is further complicated by the fact that the various species differ markedly in their susceptibility to certain poisons, with man ranking among the more susceptible. Thus, a dietary level that is safe for livestock and may even exert a favorable effect on their growth cannot be assumed to be safe for man.

Finally, in the case of those medications used to treat actual disease of animals, the doses used frequently border on the toxic. This is considered justifiable where the benefits can be expected to outweigh the possible harm. Such considerations are not applicable to the evaluation of tissue residues, since man stands to gain nothing and can only lose where the concentration is high enough to cause him injury.

When the potentialities of the problem became apparent, the Food and Drug Administration published a Statement of Policy to the effect that it regarded those compounds that were intended to affect the structure or function of the body of animals as new drugs. Furthermore, ground for refusal to make the application effective would be based on the absence of satisfactory evidence, showing that the meat or other food obtained from animals fed the drug is at the time of marketing entirely free of any poisonous or deleterious ingredient resulting from the use of these drugs. This statement was published in the *Federal Register* of December 4, 1948, and has been the basis on which the use of these products has been regulated. Since these products are not "pesticide chemicals," as that terms is used in the recent Pesticide Chemicals Amendment of the Federal Food, Drug, and Cosmetic Act, there is no provision in that amendment for establishing tolerances for them.

It is recognized that by requiring a "zero residue" this Statement of Policy opened up an analytical problem nearly as big as the public health problem that it had solved. To put it in its simplest terms, how sensitive does an analytical method have to be to establish that no residue is present? The answer has been tailored to the individual drugs that are added to feed. We have required that in addition to showing zero residue the method be sensitive enough that the traces of residue that may have escaped detection be so small that there is not even a remote possibility that they could injure the person consuming them. In some cases it has required considerable ingenuity to achieve such sensitivity.

In contrast to the medicated feeds that are used simply for an economic advantage are those that are used for the treatment (or prevention) of actual diseases. Here it is recognized that tissue residues may sometimes be unavoidable, and the requirement has been that, where a residue may remain in the tissues of the treated animals, this residue be of such an order that there is no possibility of injury resulting from the use of the products of such animals as food. Again regulation has been achieved entirely through the New Drug Section of the Act. No formal tolerances have been promulgated, but the directions on the label have been such as to limit the size of the dose, or the frequency, or the use just prior to slaughter in such a way that a safe level in tissues will not be exceeded.

In summary, it may be said that the introduction of medicated feeds has created an extensive regulatory problem for the Food and Drug Administration. It was a problem that was recognized early and has so far been handled adequately under existing legislation.

Problems Related to Drugs in Feeds from the State Control Officials' Standpoint

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The practice of controlling animal diseases through feeding rations containing drugs is of comparatively recent origin. With its development, come many opportunities and many problems that did not previously exist. To the feeder, it afforded what appeared to be a convenient and practical method of curtailing mortality, enhancing flock and herd health, and promoting growth without increasing labor costs and thereby increasing production and profits. To the manufacturer, it provided another service he could render his customer with a possible increase in feed sales. To the state control official, it imposed the obligation to require sufficient label information so the feeder would know what he was feeding and how he should feed it. Furthermore, it placed upon him the responsibility to determine accurately the presence of minute quantities of drugs. To the general public, if at all aware of the problem, it raised the question of possible contamination of the produce with harmful residues. Not one of these groups was in a position to judge if there were real hazards from the mass medication of livestock and poultry. Furthermore, the layman was to be provided with potent drugs he had previously been able to obtain only upon the prescription of a physician or veterinarian. The feeder had little knowledge and experience in the administration and use of such powerful agents. There was a tremendous obligation, therefore, upon someone to inform the feeder of the facts.

The feed manufacturer and the state feed control official had some general information they could impart, but they did not have the experience and technical background necessary to insure public safety in a medicated feeding program. Fortunately, the Federal Food and Drug Administration had had more than 50 years' experience in testing drugs and regulating their interstate shipment under the Federal Food, Drug, and Cosmetic Act, which includes drugs for animals as well as for man. Under the Federal law, the addition of drugs to feeds for the treatment or control of diseases or for the purpose of promoting growth of animals classified these articles as drugs.

Since the Federal Constitution assigns certain powers to the Federal government and intrusts other powers to the several states, it is generally acknowledged that articles of commerce passing across state lines are subject to Federal jurisdiction, while those moving only within the confines of a state come within the province of state regulations. The relationships of Federal and state jurisdictions are well recognized and have led to cooperative interplay between Federal and

state agencies. In this respect, the Federal Food and Drug Administration and the state feed control officials for many years have consulted, cooperated, and integrated their work and utilized their facilities to complement each other. The state feed laws, unlike the Federal Food, Drug, and Cosmetic Act, do not include drugs themselves; however, medicated feeds are not exempt from state regulation merely because they contain drugs. The state feed laws are essentially Acts requiring the proper identification of products. In the case of medicated feed, adequate directions for use are very important and become a part of the requirements for identification.

The feed industry developed a strong positive interest in medicated feeds after carefully conducted experiments had shown that formula feeds could safely serve as a vehicle for the administration of drugs to treat and control diseases and promote animal growth. This interest was hastened further by the popularity of such a program with the feeder. Consequently, there was a tremendous demand for medicated formula feeds.

It was at this stage that the state control officials came face to face with the problem. Fortunately for these officials, the additives came within the new drug classification of the Federal Food, Drug, and Cosmetic Act. This means that the drug manufacturer must file new drug applications listing details of formulation, data on safety, procedures for mixing, handling, and control, as well as proposed labeling and directions for use of the medicated products. This procedure provided for uniformity and standardization of labels through a central organization. The regulation of medicated feeds has been and will continue to be largely in the hands of the several states working in cooperation with the Federal government. Because of the harmonious relationship existing between the Food and Drug Administration and the Association of American Feed Control Officials, and through the excellent liaison work of L. E. Bopst, Executive Secretary of the Association, it has been possible for each control official to have, almost immediately, complete information on the drugs that were permitted in feeds as well as examples of correct labeling procedures. This service provided the control official with expert information and permitted him to accept registrations without delay to the feed manufacturer. Although we may feel that medicated feeds have presented acute problems for all of us, I shudder to think of the confusion that would have existed had we been without this channel of information.

The development of labels for interstate shipments of medicated feeds was a great convenience to both state control officials and industry. These same patterns were applied to intrastate shipments, thereby resulting in a uniform label for all medicated products. At the state level, however, there are many small mixers who operate only in a local area. They produce a large portion of the feed used on farms. Some of these mixers are not members of a state or national trade association; therefore, they do not have the advantage of expert information which may have been passed along to their larger competitors. Frequently, their chief source of information is the salesman whose scientific knowledge may be limited, but who is uninhibited in extolling the virtues of the product he sells. The local mixer may not readily see the necessity of complying with interstate requirements, since his operations are confined to a given state. He may be unfamiliar with the toxicity and possible hazards of a potent drug. In instances of this sort, the control official has to resort to a program of educating the mixer and the feeder, if compliance with the requirements is to be obtained.

May I pause at this point to ask that you not misinterpret these remarks. We did not state that the small mixer does not know what he is doing. We were merely saying it may be necessary to sell him on the idea that he has an obligation to his customers. In fact, some of our most cooperative and successful mixers of medicated feeds have been the small progressive operators. They are cognizant of their obligation to the feeder, the potential hazards from drugs, the limitations of their knowledge and facilities, and their operations.

We are aware of some criticisms that have been directed toward the current medicated feed labeling pattern. Doubtless these criticisms are justified if we use the conventional formula feed label as a standard. However, have we fully recognized the fact that a medicated feed is an entirely new product? I am sure we would agree that alcohol containing 5 per cent iodine is no longer alcohol and that water containing 1 per cent digitalis is no longer water. Potent chemicals in feeds are drugs as much as iodine, digitalis, or adrenaline are drugs in their particular vehicles. Any drug is subject to the labeling requirements of the drug laws regardless of the vehicle. I would prefer a simpler label, and I do not know that anyone would disagree with this viewpoint. But simplicity must not be obtained at the expense of safety.

The first test to be applied to a medicated feed label is that of compliance with legal specifications. This is, in a sense, also a test of safety precautions because laws and regulations have been established on the basis of experience, which has demonstrated the need for the required statements. The user of the medicated product must be told what it is in terms of the name of the drug and its potency, when and how to use it, and when not to use it. The second and equally important test applies to the label's effectiveness as a communication. Is the label in the form that renders it most likely to be read and understood by the farmer or other users? The information must be conveyed to the reader or its whole purpose is lost.

In the discussion of "simplicity," some people may have been referring to this communication problem in a broad sense. Others may have been seeking brevity alone. There is a wide spread of opinion in this area of discussion. Some of it is colored by old customs, old habits, and old concepts. However, in the present labeling of medicated feeds, we should remind ourselves that we are dealing with something new. Never before have we been confronted by practices of this character and magnitude. Both official and trade opinion can perhaps benefit by a freshened, realistic attitude in this direction.

The difficulty of conveying ideas to a reader is not unique to the field of labeling. Others have the same problems in transmitting information and instructions. There are many books on the subject, but a few simple rules may be all that we need to re-examine what we are doing or what may be proposed.

The label should, of course, be complete. It should carry all information needed for safe, effective use of the drug or medicated feed. In general, such statements are prescribed by law, but additional ones may be needed or advisable, particularly in those cases in which warnings are in order.

The label should be concise. This means that all statements should be brief and direct but with full meaning. It also means that necessary information should not be diluted or dispersed by other statements that are not positively essential.

The label should be clear. The identification must have prominence. The

directions and warnings must be in common, simple language. Here, as in all labeling, we must take the view of the user, not that of the official or the salesman. If we strive for the maximum in completeness, conciseness, and clarity, and with a full sense of our responsibility, we will do the job as it should be done. Other matters that have troubled us at times will subordinate themselves and permit easy accomplishment of our goal—the most effective labeling of all medicated feeds.

Medicated Feed as an Economic Factor in the Livestock Industry

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The medication of feedstuffs is one of a group of recent feeding innovations that have important economic implications. These technical changes in feeding practice are improving the efficiency of feed conversion, that is, the pounds of livestock product produced per 100 pounds of feed. They also affect the organization of production on the farm.

Within only a few years, a rather large number of substances have come to be added to livestock feeds—they are sometimes spoken of as "additives." Only a few of them are medications; the rest include such substances as vitamins, antioxidants, urea, trace minerals, and unidentified growth factors. They usually appear in rations in various combinations. Economic implications therefore can best be discussed in terms of the general effects of this whole group of technical innovations.

For many years, the average rate of feed-conversion efficiency—the pounds of feed used to produce a given quantity of livestock—has changed very little. A feed unit is a quantity of feed in which the actual weight of each feed is adjusted to reflect its feeding value in comparison with corn. From 1926 to 1929, 1.53 tons of feed units were consumed per $100 of livestock produced (at 1947–1949 values, with horses and mules excluded). From 1950–1953, 1.52 tons were used to produce the same volume of output (table I). Over this period, marked improvements were made in livestock output per animal and in rapidity of gain. But higher producing animals eat more feed per day as a result of increased capacity and the feeding of rations that are more concentrated and better balanced. It is generally accepted that as the daily rate of feeding is increased, a point will be reached beyond which output per pound of feed will decline. Except for poultry, this tendency toward diminishing physical returns may have offset almost entirely the gains in efficiency of feed conversion that have resulted from improved breeding, nutrition, and sanitation. It should also be mentioned that the protein content of corn has declined somewhat.

It appears that the new developments in livestock feeding that are just coming into use may result in substantial gains in livestock production per feed unit. Marked changes have already taken place in poultry feed requirements. About 4.2 lb. of feed were used to produce 1 lb. of broiler (live weight) in 1941. By 1954, the amount of feed had declined to about 3 lb., a reduction of more than 25 per cent (see table II).

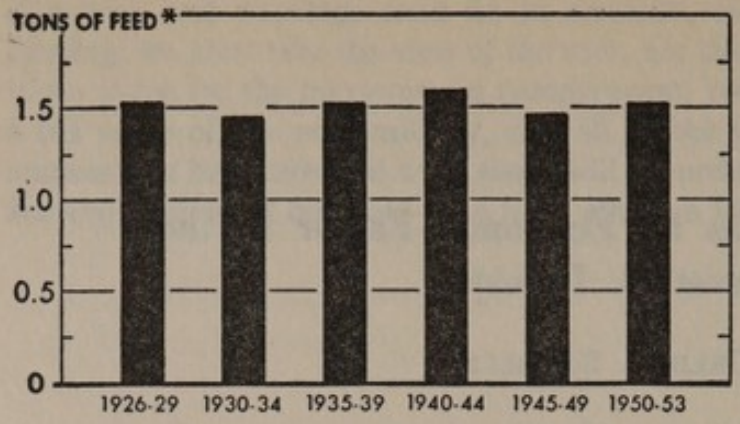

FIG. 1. Change in total feed-conversion efficiency.

* Per 100 dollar livestock output at 1947 to 1949 prices.

Under experimental conditions, broilers have been produced on less than 2 lb. of feed/lb. of broiler. These gains in feed efficiency are due partly to improved breeding, but innovations in feeding are responsible for much of it. Similar innovations in feeding are being introduced for other classes of livestock. Apparently, we may be on the threshold of a new technological breakthrough that may be more significant to future production than was the introduction of hybrid corn. If so, what are some of the implications to agriculture? What will be the effect on costs of production? How will the comparative advantage among livestock enterprises be affected? What will be the effects on farm organization and scale of production?

Medicated feeds may affect production costs in three ways: (1) increased output per pound of feed, (2) more rapid gains, and (3) change in quality of product.

The economic significance of these effects can be illustrated by data from a Connecticut broiler study.[1] In this study, the addition of an antibiotic plus vitamin B_{12} increased output per pound of feed about 12 per cent and reduced the time required to produce a 3.6 pound broiler by 8 per cent. In addition to the saving

TABLE I

Feed Consumption and Feed-conversion Efficiency

Period	Feed fed per year, in feed units*				Livestock production at 1947-49 farm values, billion dollars†	Tons of feed per $100 livestock output
	Concentrates, million tons	All feeds, million tons	Proportion concentrates, per cent	All feeds except feed fed to million tons workstock,		
1926-1929	105.7	255.6	41	199.5	13.00	1.53
1930-1934	94.7	241.3	39	196.1	13.61	1.45
1935-1939	93.5	238.6	39	199.2	13.25	1.52
1940-1944	127.2	303.9	42	267.6	16.83	1.59
1945-1949	122.5	285.1	43	256.6	17.46	1.47
1950-1953	127.5	293.0	44	273.8	18.09	1.52

* U.S.D.A. Circular 836, Consumption of Feed by Livestock, 1909-47, 1949, and unpublished data from the Production Economics Research Branch, Agricultural Research Service, United States Department of Agriculture.

† United States livestock output going into consumption use. U. S. Department of Agriculture Handbook 91, Measuring the Supply and Utilization of Farm Commodities, November 1955.

TABLE II

*Relationship between Feed Consumed and Broiler Meat Produced**

Year (beginning Oct. 1)	No. lb. feed consumed/lb. of broiler produced (live wt.)
1940	4.21
1945	4.07
1950	3.37
1953	2.96

* Unpublished data from Production Economics Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

in feed costs, labor and overhead costs were also reduced as a result of the time saved in production. Net return per bird was increased from $0.12 to $0.19, or 58 per cent. If the time saved as a result of more rapid growth were used to produce additional lots of broilers, the net effect of this feed innovation would be a potential increase in annual income of about 67 per cent.

With other classes of livestock, a moderate improvement in feed efficiency would not affect profits so spectacularly because rate of growth would not be so important a factor. But even if the only effect of the feeding innovation is a moderate reduction in feed costs, this may result in a much higher percentage increase in profits, at least in the short run.

Changes in feed technology have already had a marked effect on the organization and operation of poultry farms, and changes are beginning to appear in other livestock enterprises.

The introduction of vitamins in poultry rations in the 1930's made it possible to keep poultry indoors on a year-round basis. With the development of complex formula feeds, the job of mixing feeds and the planning of rations began to shift from farmers to the feed industry. Farmers are using more and more formula feeds for all classes of livestock. Production of manufactured feeds now is around 35,000,000 tons a year, compared with about 9,000,000 from 1930 to 1934.

Broiler production is one of the outstanding "growth industries" of today. In 1934, 34,000,000 commercial broilers were produced in the United States. In 1954, the number was more than a billion.

The commercial broiler enterprise owes much of its rapid development to the mixed-feed industry and the services associated with it, including managerial

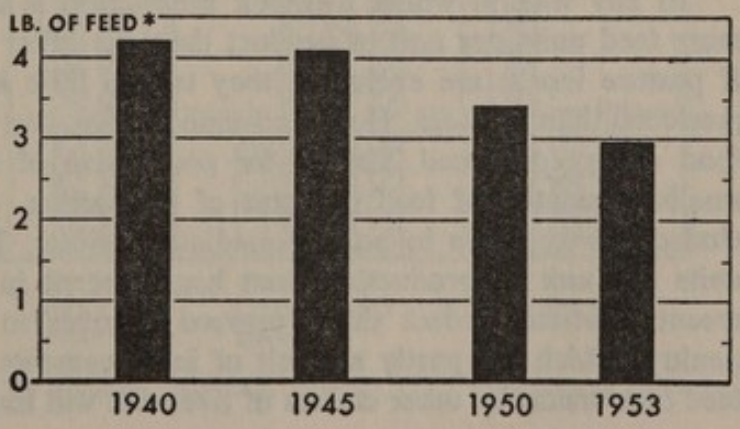

FIG. 2. Increase in feed-conversion efficiency in broiler production.

* Per pound of broiler produced.

TABLE III

*Feed-conversion Efficiency, by Species, 1949-1953**

Class of livestock	Feed units consumed per 100 lb. live weight	100 lb. meat and fat, not bone	100,000 calories (food energy)	Pound of protein
Including pasture input				
Hogs	515	819	324	95
Cattle and calves	952	2186	1552	138
Broilers	359	674	834	34
Turkeys	543	885	740	44
Excluding pasture input				
Hogs	490	779	309	90
Cattle and calves	577	1325	940	84
Broilers	359	674	884	34
Turkeys	518	828	706	42

* Unpublished data, Production Economics Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

advice and credit. A recent South Carolina study showed that from a third to more than half of the farmers interviewed delegated to feed dealers important management decisions relating to choice of ration, choice of breed, when and to whom to sell, and what price to ask.[2]

It is not likely that the effects of innovations in feeding will be as spectacular with other classes of livestock, but the preparation and mixing of feed will shift from farms, and managerial decisions on livestock rations will be shared with feed manufacturers and distributors. Possibly, credit arrangements similar to those in effect for broilers may be developed by feed distributors, though not to the same extent. In many broiler areas, opportunity to expand broiler production through liberal credit arrangements has been facilitated by the fact that farmers have had low costs for their labor.

To the extent that feeding innovations are more productive for one class of livestock than for another, farmers may be expected to shift farm enterprises to maximize returns. Recent figures on feed units consumed by different classes of livestock per unit of production are of interest (table III). Feed-conversion efficiency is here measured in terms of production of live weight, meat and fat, energy, and of protein.

In any way in which livestock production is measured, beef cattle consume more feed units per unit of product than the other classes of meat animals listed. If pasture inputs are excluded, they take a little less feed per pound of protein produced than do hogs. Hogs consume the smallest quantity of feed per calorie of food energy produced. Except for production of food energy, broilers take the smallest quantity of feed per unit of production, even if pasture inputs are excluded. Turkeys are in an intermediate position. They use somewhat more feed units per unit of production than hogs, except in production of protein. These recent estimates reflect sharp upward changes in feed-conversion efficiency by poultry, which are partly a result of improvements in rations. It is probable that feed conversion by other classes of livestock will improve relative to poultry in the

next few years, but poultry may retain some advantage. To a greater extent than in the past, broilers are likely to compete with hogs for farm resources.

Improvements in feed technology and other developments that have accompanied it, including breeding, have profoundly affected the scale of broiler production. This enterprise has tended to move toward a specialized industry with the scale of production organized around full utilization of the family labor supply. Land and, usually, working capital are no longer restrictions on scale of operation. Risks are shared and therefore have less influence than formerly. Size is still family-scale, but a family can handle more birds. In 1945, one man could, in a year, care for about three lots of broilers with about 6000 in each lot, or 18,000 broilers for the year. Now a good standard would be four lots of 8000 each, or 32,000 birds.

Developments in feed technology are likely to affect the scale of other livestock enterprises to some extent, although not so much as for broilers. There will probably be some expansion in big "assembly-line" feeding operations, but feed innovations should not place small feeding operations at a disadvantage. In fact, making technical knowledge about the compounding of rations available to anyone who buys a bag of feed should help small feeders to compete. Faster rates of gain mean that livestock are kept fewer days and therefore require less labor. The labor saved may go into increased livestock production. Similarly, improved feed efficiency will permit a larger number of livestock to be fed with given feed resources. The net effect will be a tendency for livestock enterprises to become larger.

Changing feed technology may have influenced the geographic distribution of broiler production. Although numbers have increased in all areas, the most rapid increase has been in the South. So far as other classes of livestock are concerned, it is difficult to say what the effects will be in different producing areas.

Improvements in feeding will increase output, and any output-increasing innovation raises a question as to who will get the benefits. In the short run, in first adopting an innovation, farmers reduce their costs and increase output and profits. But as the new technique becomes generally adopted, the price of the product may decline if demand for livestock products is somewhat inelastic, which may be the

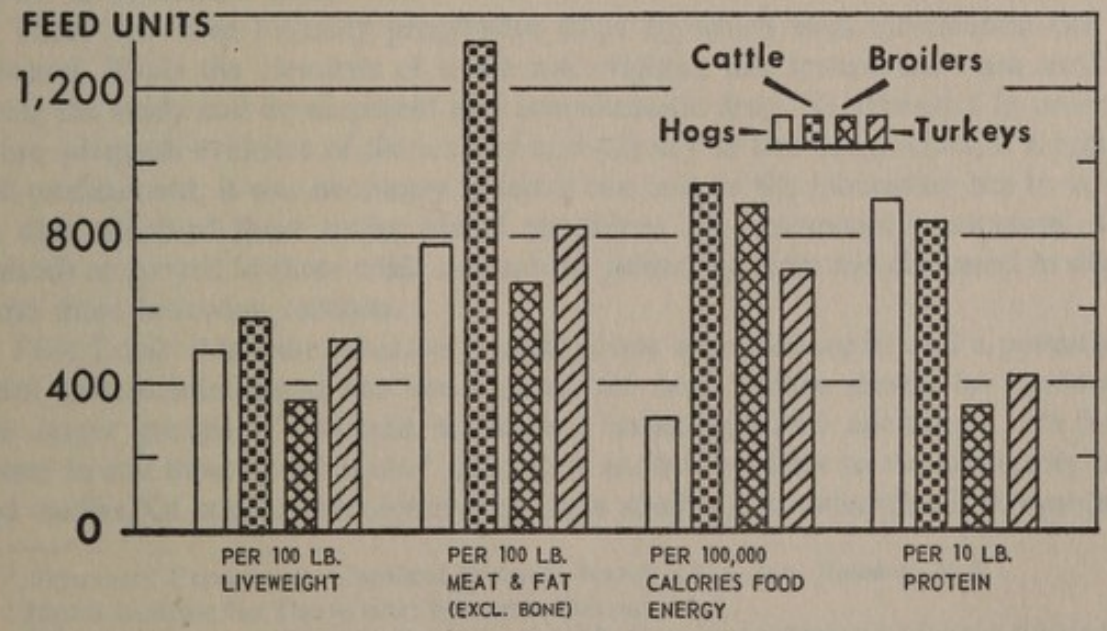

FIG. 3. Feed-conversion efficiency by kinds of livestock, 1949 to 1953, exclusive of pasture.

case, at least in the short run. However, this effect may be offset by the continual growth in consumer demand because of rising population and other factors. The situation would vary considerably among producers of various kinds of livestock.

Since antibiotics, stilbestrol, and other feed additives reduce the amount of feed required per unit of livestock production, rapid and widespread introduction of these substances might reduce the demand for feed grains enough to cause a short-run decline in the incomes of producers of feed crops. In time, livestock production would probably increase about in proportion to the increase in feed efficiency, so that total demand for feeds would be restored.

In summary, we appear to be on the verge of a significant technologic improvement in livestock feeding. Introduction of medicated feeds and other feed additives are a part of this development. Recent advances in poultry nutrition, particularly for broilers, indicate some of the possibilities and also suggest that the rate of technologic change has speeded up since the time of hybrid corn. Improvements in feeding technology will tend to increase specialization in livestock production, will increase the scale of enterprises somewhat, and may cause some shifts in production among enterprises and regions. Certain operations and management decisions will be shifted away from the farm.

Innovations in feeding will lower costs and expand output. Incomes will be improved for those who first adopt these innovations but, with widespread adoption, prices may fall and much of the gain from the innovation will probably accrue to consumers.

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Suggested Pattern for Field Evaluation of Medicated Feeds

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It has long been an axiom that, before any drug can be offered for public sale, its usefulness must first be properly assessed. First, the preparation must be safe (e.g., nontoxic) when used in accordance with the techniques of administration and in the amounts recommended by its manufacturer. Secondly, but no less important, the compound must be efficacious. Further, in the case of a drug intended for use in livestock, its efficacy must be such as to permit its use at economic levels. This paper is concerned with establishing the second point.

The techniques usually employed in the laboratory can clearly define tolerance for a drug in a given species. They can also determine effective doses of the substance for artificially produced cases of the condition it is desired to treat. Beyond this, the physical limitations imposed by ordinary laboratory facilities make it difficult to progress further. In human medicine, drugs are taken to various teaching hospitals or other institutions where investigative programs are carried on. There, final evaluation of efficacy can be made on natural cases of the disease. A potential veterinary preparation, especially one that is designed for addition to feed or water for the treatment of large numbers of animals, requires a similar opportunity whereby performance of the medicament can be assessed. It must be taken into account that the drug must be effective under the varied methods of husbandry and conditions of equipment, housing, and weather, and against the disease in its natural form.

There are three logically progressive steps by which such information can be collected. While the elements of it are not original, this system has been evolved during the study and development of a coccidiostatic drug, Nicarbazin.‡ In order to secure adequate evidence of the activity and efficacy of this drug, which is a typical feed medicament, it was necessary to test it not only in the laboratory but to verify the data obtained there under actual conditions of commercial production. The methods employed in these trials and results gained by them are discussed in detail in the three following sections.

Pilot Trials. After the laboratory studies have been performed and a potentially useful anticoccidial agent has been found, the next studies should be conducted with larger groups of chickens maintained under practical conditions. We have chosen to call these "pilot trials," since they are intermediate to the laboratory and field studies. Of course, the objective of these studies is to determine if the product

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‡ The trade name of Merck & Co., Inc., for 4,4'-dinitrocarbanilide·2-hydroxy-4,6-dimethylpyrimidine complex is Nicarbazin.

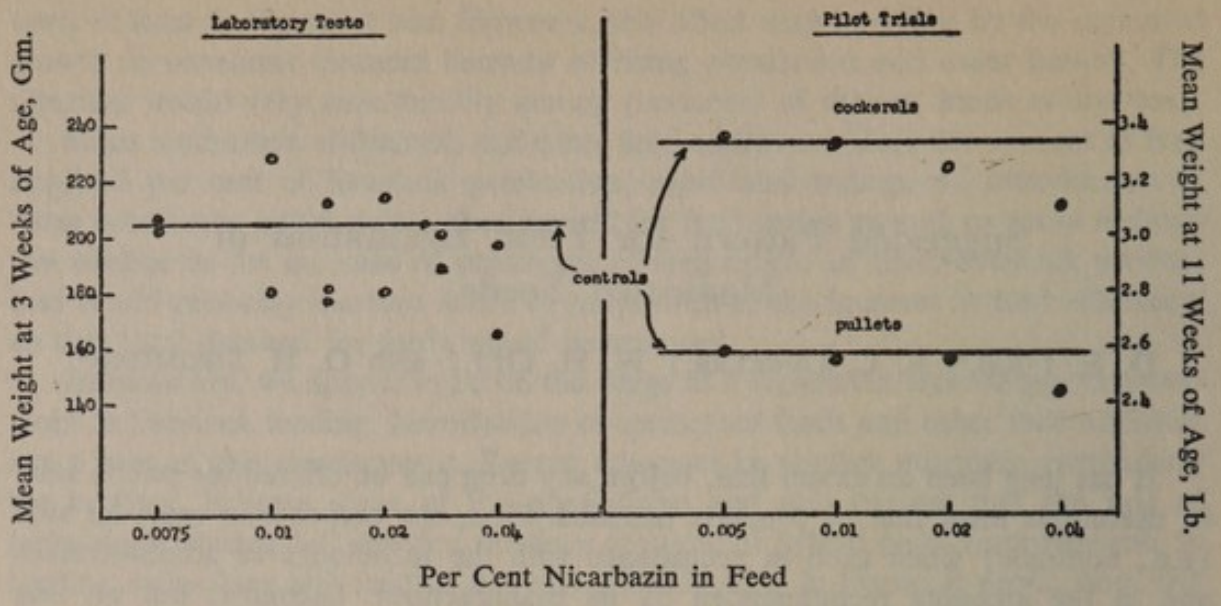

FIG. 1. Effect of nicarbazin on growth. — = Controls; o = nicarbazin.

under evaluation will perform as satisfactorily in these circumstances as in the laboratory trials. In addition to the observations on anticoccidial efficacy, one can determine what effect the compound has on growth, feed efficiency, and maturation of the chickens. During the course of these studies, compounds that appeared to be satisfactory coccidiostats under laboratory conditions were shown to be toxic after prolonged feeding under pilot trial conditions. Obviously, such compounds are not commercially attractive and it is necessary that this information be uncovered as quickly as practicable.

For our pilot trial studies, we have used poultry houses of two types. In one, the house is divided into 10 similar pens, each accommodating 155 chickens. The other houses have four pens, each of which handles 800 chickens. The smaller house is generally used when several replications are required or when comparative results are sought. These facilities have been very useful also for establishing the relative efficacy of graded concentrations of a feed additive. The larger houses of four pens each have been useful for replicating one level of an anticoccidial agent or getting data on the compound under more nearly commercial conditions.

The following descriptions of experiments are examples of the manner in which these facilities have been used to determine whether nicarbazin, a new anticoccidial agent, was as efficacious and safe as laboratory studies had suggested.

Tolerance studies were conducted with groups of young chicks kept in laboratory battery brooders and fed graded concentrations of nicarbazin for three weeks. The effect of nicarbazin on the growth of young chicks under these acoccidial conditions is shown in figure 1.

For comparison, the results obtained in pilot trials are also shown in this same figure. There were five replicate floor-pen trials with graded concentrations of nicarbazin and artificial exposure to coccidiosis. The growth of the chickens was observed for 11 weeks. The results obtained are in complete agreement with the laboratory trials. The data indicate that in both acoccidial and coccidiosis-contaminated environments, chickens grow normally when fed concentrations of nicarbazin up to 0.03 to 0.04 per cent for 3 or 11 weeks.

A comparison of anticoccidial efficacy under laboratory and floor-pen conditions is shown in figure 2. These data indicate that there is general agreement between the laboratory battery brooder trials and the floor-pen trials. Lest the coccidiosis mortality figures in the nicarbazin-treated group cause alarm, it must be recalled that these chicks were infected directly by oral administration of large numbers of oocysts.

The comparative results obtained with nicarbazin and three competitive coccidiostats in five replicate floor-pen experiments are shown in figure 3. These data further demonstrate the anticoccidial effectiveness of nicarbazin.

The results that we have presented demonstrate that the laboratory and pilot trials with nicarbazin are in complete agreement. However, exceptions of an important nature have been noted. These occurred where, on the basis of long-term feeding, certain therapeutically effective anticoccidial agents were found to interfere seriously with the normal growth of chickens. We believe that both types of investigation should be employed and that pilot trials are necessary before going to the more extensive field trials in the evaluation of medicated feeds.

Controlled Commercial Trials. While the pilot trials provided important data on the effects upon the chickens themselves of the drug under study, the influence upon nicarbazin activity of factors involved in actual production for market could not be accurately gauged. The numbers of birds used, while considerably larger than laboratory battery studies, did not approach the many thousands of birds raised in a market broiler plant. It became obvious that a facility had to be secured that was not only adequate for full-scale broiler production under accepted commercial conditions, but also in which close control of every phase of production could be exercised.

Accordingly, a complete broiler production farm, including houses for 30,000 birds, a feed storage building, heating equipment, and other necessary feeding and watering equipment, was leased and staffed with our own personnel, under professional supervision. All operations were conducted in a manner commonly

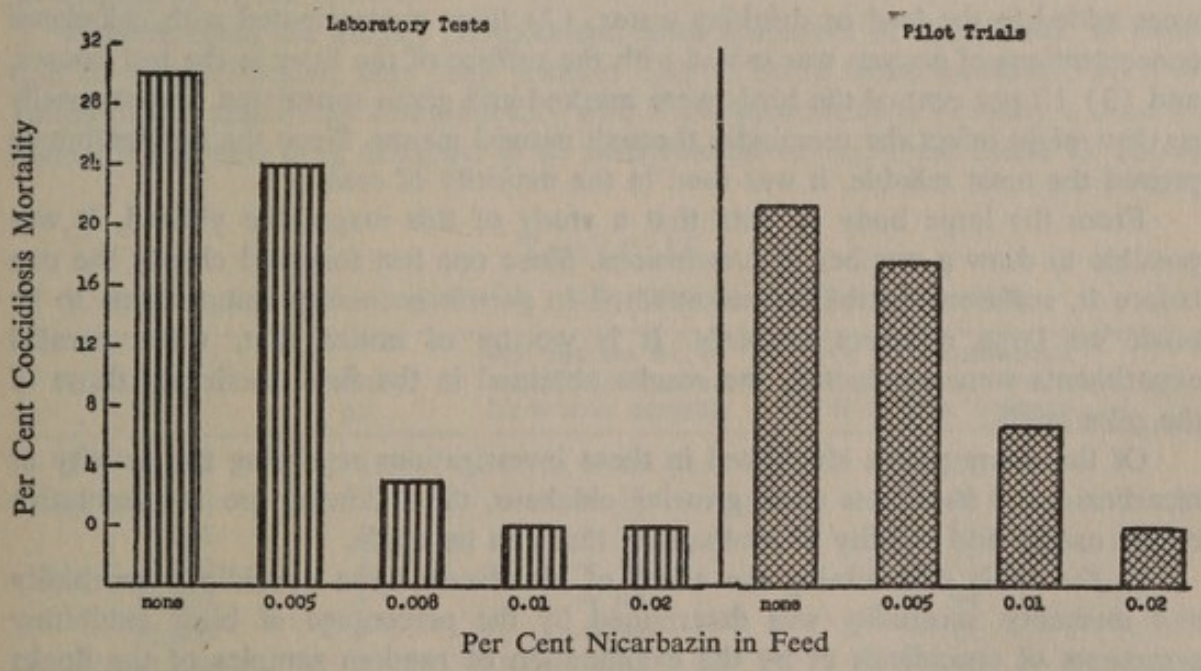

FIG. 2. Effects of nicarbazin on coccidiosis mortality from *Eimeria tenella*.

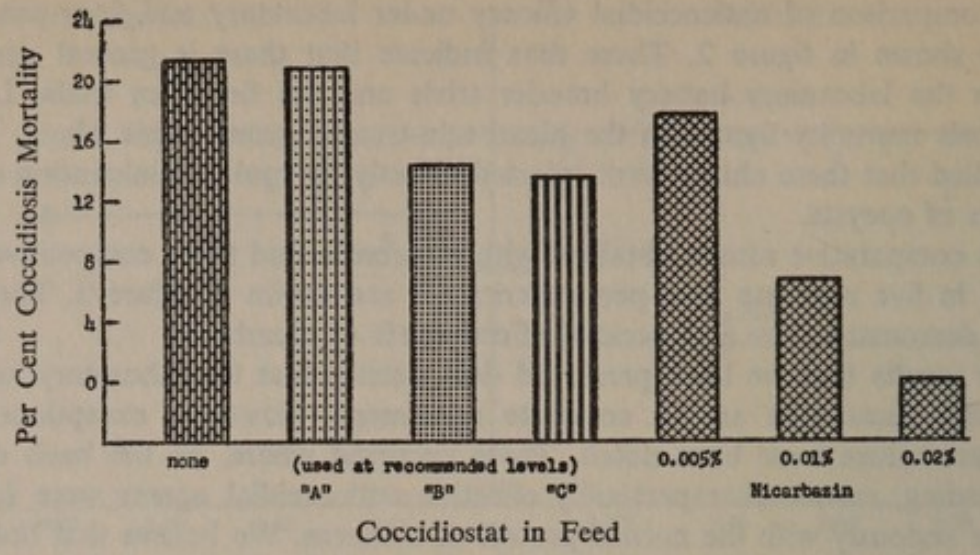

FIG. 3. Comparison of effects of several coccidiostats on coccidiosis mortality during pilot trials.

employed in broiler production, with the exception of feed mixing and the detail in which records were kept. While a standard brand of feed was used, nicarbazin and other additives tested were custom-blended under supervision. Samples of these prepared feeds were frequently assayed for drug content.

In order to maintain control over the entire trial, all feeding and watering were done by personnel trained in laboratory techniques and familiar with chickens. Adequate controls were established for all experiments (each of which comprised from 16,000 to 19,000 birds) and color-coded feed bags were used to safeguard further against errors in feeding, particularly if comparisons with other drugs were being made. All experimental groups were replicated four times, except in a few special instances. In only one respect did these trials depart from a normal broiler production venture; this was in the means of positively inducing coccidiosis infection. Three methods were used: (1) predetermined numbers of sporulated oocysts were added to the feed or drinking water, (2) litter contaminated with calculated concentrations of oocysts was mixed with the surface of the litter in the test houses, and (3) 10 per cent of the birds were marked and given sporulated oocysts orally so they might infect the remainder through natural means. Since the first technique proved the most reliable, it was used in the majority of cases.

From the large body of data that a study of this magnitude yielded, it was possible to draw a number of conclusions. Since one test followed closely the one before it, sufficient flexibility was attained to permit necessary comparisons to be made on large numbers of birds. It is worthy of notice that, when parallel experiments were conducted, the results obtained in the field confirmed those of the pilot trials.

Of the many points elucidated in these investigations regarding the activity of nicarbazin and its effects upon growing chickens, the following are representative of the nature and validity of evaluations that can be made.

In the trials determining the effect of nicarbazin upon coccidiosis morbidity and mortality, morbidity was determined by the percentage of birds exhibiting symptoms of coccidiosis or by the examination of random samples of the flocks for gross lesions of coccidiosis. The reduction in both morbidity and mortality to

TABLE I

Elimination of Coccidiosis Mortality by Nicarbazin

Field trial	Nicarbazin conc.in feed, %	Mortality (in %)		Morbidity* (in %)	
		Control	Nicarbazin	Control	Nicarbazin
A	0.010	1.04	0	†	†
B	0.0125	10.90	0	73	0
C	0.015	3.94	0	27	0

* Number of birds showing symptoms or gross lesions.
† No record kept.

zero in field cases of coccidiosis is apparently a fixed characteristic of nicarbazin (table I). A further confirmation of this will be seen in the later reports on trials on farm flocks.

While mortality prevention is an extremely important feature of a drug's activity, it must also do this economically. One measure of economic performance is the growth and feed conversion of treated birds in comparison with untreated controls. The results of such a study are seen in table II. The nicarbazin-treated birds show significant increases in gains over the controls. Although the market weight of control birds in trial 7 exceeds that of those fed nicarbazin, the latter possessed a feed-conversion ratio sufficiently superior to that of the controls so that they were quite as profitable as the other groups.

Naturally, the activity of any new coccidiostat as compared with those already on the market is a prime question. Battery and pilot studies had already shown nicarbazin to be more active than other drugs and the results of the field trial corroborated them. Table III summarizes the data obtained from this experiment. Not only mortality, but a number of other economic factors, such as number of culls, weight gains, feed conversion, and profit at marketing are also recorded. The comparative value of any given coccidiostatic drug can be easily assessed by an inspection of the figures.

Modern feeds are usually compounded from a number of ingredients. In addition to a coccidiostat, they may contain one or more other additives, such as antibiotics or arsenicals. Compatibility with these substances is virtually a requirement for any new drug designed to be administered via the feed. Table IV shows

TABLE II

Effect of Nicarbazin on Market Weight and Feed Conversion

No. birds	Trial no.	Av. market wt. of nicarbazin*-treated birds over controls	Av. feed conversion of nicarbazin*-treated birds over controls
16,000	1	+.19	−.24
16,000	2	+.19	−.31
5,000	3	+.08	−.17
11,000	4	+.12	−.45
18,000	5	+.02	−.04
12,600	6	+.25	−.12
12,240	7	−.21	−.40

* Nicarbazin fed either at 0.0125, or 0.015 per cent.

TABLE III

*Comparison of Nicarbazin with Other Prominent Coccidiostats**

	Control	0.0125% Nicarbazin	0.0055% NFZ	0.02% NPH	0.2% Polystat	0.0175% Sulfaquinoxaline
No. birds started	4200	3200	3200	3200	3200	1400
Average weights	2.61	2.87	2.54	2.51	2.66	2.63
Average feed conversion	2.92	2.61	3.00	2.80	2.72	2.81
Coccidiosis mortality (%)	6.2	0	10.04	0.85	3.67	3.00
Culls unsalable (birds)	32	12	72	22	17	28
Return over feed and drug costs per bird (¢)	20.6	29.4	12.2	22.3	24.3	24.3

* Figures are averages of four replicates.

the results of a test designed to determine the compatibility of nicarbazin with such common feed additives. It is readily obvious that not only does nicarbazin evidence compatibility, but also that it enhances the performance of birds fed the supplemented rations.

Laboratory studies had shown that the optimum concentration of nicarbazin lay between 0.01 and 0.02 per cent of the ration. To establish definitively a dosage for general use, several experiments were carried out under commercial conditions using smaller numbers of birds in each group. The results of these trials are given in table V. Under the conditions of this study, the most economical concentration of nicarbazin is clearly 0.0125 per cent. Pilot trials had previously given the same results.

Field Trials in Farm Flocks. The third phase of this evaluation program abandons the techniques of the carefully controlled trial and places the product, prepared as a medicated feed, into use on a number of working chicken farms. This procedure allows the many variations of management practices, weather conditions, and physical equipment to exercise their influence on the performance of a feed additive. In such tests, controls can seldom be maintained, for obvious reasons. Accordingly, in the tests conducted on these farms, controls were only

TABLE IV

*Effects of Nicarbazin, an Arsenical, and Antibiotics Added to a Commercial Ration**

	Control	Nicarbazin, 0.0125%	Nicarbazin + arsenical†	Nicarbazin + penicillin, 25 Gm./ton 3 days/wk.	Nicarbazin + penicillin, 25 Gm. streptomycin, 75 Gm./ton 3 days/wk.
Average weight (lbs.)	2.47	2.60	2.69	2.78	2.77
Average feed conversion	3.50	3.19	3.09	3.09	3.01

* Figures are average of four replicates.
† 3-Nitro-4-hydroxyphenyl arsonic acid.

TABLE V

Determination of Optimal Use Level

N.C. drug level, %	No. started	No. sold	Total mort., %	Coccidiosis (*E. tenella*) Mortality	Coccidiosis (*E. tenella*) Morbidity	CRD mort., %	Culls	Price /lb., ¢	Cost /1000 sold, $	Rec'd. /1000 sold, $	Profit, $
0.01	600	576	5.8	0	0	3.1	4	31	515.00	657.70	142.70
0.0125	700	685	3.0	0	0	2.3	6	30	427.50	822.50	395.00
0.015	500	408	9.6	0	0	8.3	2	30	520.40	811.00	290.60
Control	1000	871	12.1	6.3	50	4.0	25	22	480.00	525.60	45.60

occasionally kept, and the use of large numbers of birds and objective comparison of experience were substituted.

It was again apparent that data obtained in laboratory and pilot studies, and confirmed in the controlled field trials, were supported further by the results obtained from these farm flocks. Two typical reports are summarized in tables III and IV. These data show the scope of the tests carried out on many flocks of varying size, comprising an imposing total of broilers.

In table VI is presented data from 36 separate flocks, comparing mortality data from groups fed nicarbazin, medicated controls that received several other

TABLE VI

Summary of Field Trial Mortality Results with Nicarbazin, Other Coccidiostats, and Controls

Nicarbazin conc. in feed, %	Lots	Chickens fed nicarbazin	Control chickens: Medicated	Control chickens: Non-medicated	Total chickens	Results
0.0055	2	1,500	—	2,000	3,500	Equal coccidiosis incidence in all groups
0.010	9	55,177	40,000	2,000	97,177	No coccidiosis in nicarbazin groups; 0 to 12.5% in controls on other medication; 5% in nonmedicated controls
0.0125	18	174,542	48,500	6,000	229,042	No coccidiosis in nicarbazin groups; medicated controls, 0 to 12%; non-medicated controls, 3 to 17.5%
0.0150	5	19,909	—	6,250	26,159	No coccidiosis in nicarbazin groups; 0 to 4% in controls
0.020	2	1,500	—	2,000	3,500	No coccidiosis in nicarbazin groups; 1.9% in controls
Totals	36	252,628	88,500	18,250	359,378	

TABLE VII

Summary of Results of Farm Flocks Fed 0.0125 Per Cent Nicarbazin Continuously Until Marketing

Flock	No. started	No. sold	Mortality (%)	Av. wt.	Feed conversion	Coccidiosis	
						Mortality	Morbidity
1	16,000	14,992	6.30	3.150	3.360	0	0
2	14,500	13,526	6.71	3.179	3.320	0	0
3	9,500	9,260	2.52	3.097	3.130	0	0
4	6,500	6,072	6.58	3.120	3.259	0	0
5	8,000	7,952	0.60	3.310	3.310	0	0
6	4,000	3,856	3.60	3.350	3.196	0	0
7	3,000	2,898	3.40	3.286	3.149	0	0
8	15,000	14,296	4.69	3.170	3.374	0	0
9	3,200	3,024	5.50	3.450	3.319	0	0
10	22,500	22,288	0.94	3.390	3.135	0	0
11	3,600	3,426	4.88	2.990	3.108	0	0
Av. Totals	105,800	101,590	4.16	3.240	3.240	0	0

coccidiostats, and birds receiving no medication. It will be observed that nicarbazin-treated birds showed no coccidiosis mortality, while both medicated and nontreated controls showed a coccidiosis mortality ranging from 0 to 17.5 per cent. Also pointed out is the fact that in the flocks fed the lowest level (0.0055 per cent) of nicarbazin, no effect on coccidiosis mortality was seen, the losses being as great as the controls. These results substantiate those seen earlier in table I.

In table VII, a general collection of data taken from flocks without controls is presented. Those factors of production on which figures are usually kept as part of ordinary maintenance of records are assembled here, and the averages, as well as the range of values, are compared with previous experience in these flocks.

From this body of data obtained from 11 flocks and comprising more than 100,000 broilers, it will be obvious that no evidence of coccidiosis was seen. Whatever mortality was reported occurred either during the first week of life or following Newcastle disease vaccination. No symptoms suggestive of coccidiosis were seen.

It had been the usual thing for each of these flocks to be treated for coccidiosis once or, more often, twice during the growing period, and to experience a coccidiosis mortality ranging from 5 to 10 per cent.

CONCLUSIONS

1. An effective method for field evaluation of medicated feeds has been evolved. It consists of three phases beyond laboratory studies: (1) the pilot trial, using laboratory techniques under conditions approaching those seen in the field, (2) the full-scale field trial, in which a commercial operation is conducted under a carefully controlled system, and (3) trials in commercial broiler operations, with few controls, but depending on large numbers for valid information.

2. This method can yield useful and correct data on the suitability of a drug as a feed medicament, as well as efficacy, optimal dosage, economy, and its performance in comparison with similar agents.

3. The three steps in this pattern are a logical progression of tests. Each step confirms data obtained in previous studies, but under a new set of conditions. Where such confirmation is not obtained, careful examination of the data may reveal lack of suitability of a compound for use in medicated feeds.

4. A preparation that successfully emerges from this pattern of tests will possess the characteristics of an effective, dependable, and economically useful feed medicament. Nicarbazin is offered as an example of a substance that fulfills this condition.

Arsenicals in Feeds

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Arsenicals have been used in medical practice for hundreds of years. Fowler's solution, containing 1 per cent arsenic trioxide, a cheap but toxic form of inorganic arsenic, was first used as a tonic to improve the appearance and well-being of men and animals alike. The toxic properties of arsenic trioxide, however, greatly limited its use, except as a general tonic in veterinary practice.

At the turn of the century, arsenic in the form of arsanilic acid and its water-soluble salt, sodium arsanilate, was found to be only one-fortieth as toxic as arsenic trioxide. Sodium arsanilate, known then as atoxyl, was reported in 1907 to cure spirochetosis in chickens. Henceforth, through the work of Paul Ehrlich and others, various derivatives of arsanilic acid became the first "magic bullets." Organic arsenicals, between 1910 and 1945, provided the best tools against man's most serious parasitic diseases.

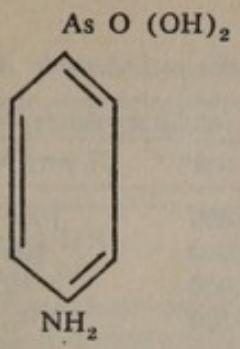
We have attempted elsewhere to tell the story behind arsenicals in feeds,[1, 2] but much remains to be told and to be learned. The tonic effect of arsenic itself remains a mystery even though it was probably one of the earliest nutritional effects recorded.

Penicillin in the early 1940's largely displaced the organic arsenicals as specifics for venereal disease. At this same time, however, Morehouse and Mayfield[3] found certain intermediates used in arsenical manufacture to be not only coccidiostatic, but growth-promoting, as well. This finding coincided with discovery of the growth-promoting effects of antibiotics and was fully verified by Bird, et al[4] in 1949. Arsanilic acid came into the picture in the early 1950's as a general growth promotant and as a specific against bloody dysentery.[1] 4-Nitrophenyl arsonic acid was introduced as a preventive for blackhead[5] and, more recently, arsenosobenzene as a cecal coccidiostat.

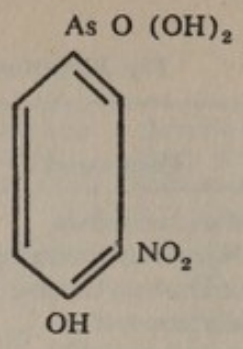
FORMULAS AND NOMENCLATURE

According to Chemical Abstracts' preferred chemical nomenclature, the arsonic acids are derivatives of benzene and are therefore most appropriately named as benzene arsonic acids, rather than phenyl arsonic acids. We have chosen to use the latter form here because it is more commonly used and understood.

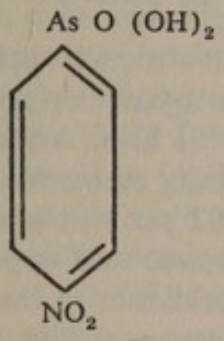
Following are formulas of arsenicals accepted for feed use:



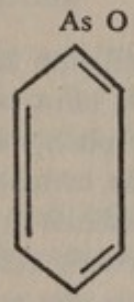
Arsanilic Acid
(*p*-aminophenyl arsonic acid or
4-aminophenyl arsonic acid)



3-Nitro-4-hydroxyphenyl
arsonic acid



4-Nitrophenyl arsonic acid



Arsenoso benzene
(phenyl arsenoxide)

The December, 1952, issue of Feed Age rounded up information on "Arsonic Compounds in Feeds."[6] This helped broaden understanding among feed manufacturers. The name "arsonic compounds" was coined to cover chiefly arsonic acids. At that time, arsanilic acid, 3-nitro-4-hydroxyphenyl arsonic acid, and 4-nitrophenyl arsonic acid were the only arsenicals used in feeds. The term "arsonic compounds" served its purpose at the time but does not have an exact chemical connotation. To avoid confusion in the future, this term should probably be dropped in favor of more exact terminology. This is particularly true because arsenosobenzene, not an arsonic acid, is now accepted for feed use.

3-Nitro-4-hydroxyphenyl arsonic acid is often referred to as "arsonic acid." This usage is seen in the official reports of some of the state feed control laboratories. It should be realized that arsanilic acid is also an arsonic acid chemically, and so is 4-nitrophenyl arsonic acid. The difference between them comes in the different substitution in the benzene ring. The name arsanilic acid derives from the fact that the compound is a derivative of aniline. It is analogous in structure to *p*-aminobenzene sulfonic acid, commonly known as sulfanilic acid. It is also analogous in structure to *p*-aminobenzoic acid and to sulfanilamide.

Chemical name	*Abbreviation*	*Trade name*
3-Nitro-4-hydroxyphenyl arsonic acid	3-Nitro	3-Nitro
4-Amino (or *p*-amino) phenyl arsonic acid	Arsanilic acid	Pro-Gen
4-Nitrophenyl arsonic acid	None commonly used	Histostat
Arsenosobenzene (phenylarsenoxide)	None commonly used	Arzene

TABLE I

The Bacteriostatic Value of Antibiotics and Arsonic Acids

Compound	Minimal inhibitory concentration, γ/ml.	
	*E. coli**	*Cl. perfringens*
Sodium arsanilate	10,000	1000
3 Nitro-4-hydroxyphenyl arsonic acid	7,000	100
p-Chlorphenylarsonic acid	7,000	10
Chlortetracycline	3–100	.01–.02
Procaine penicillin		0.08

* Representative of *Str. faecalis, P. vulgaris,* and *S. enteritidis.*

RELATION OF STRUCTURE TO ACTIVITY

No clear correlation has developed between structure and growth-promoting effect. Compounds most potent as bacteriostatic agents are also most effective as coccidiostats. The phenyl arsenoxides head the list here. Arsenoso benzene is the most potent of the commercial coccidiostats, being recommended for prevention against cecal coccidiosis in chickens at only 0.002 per cent of the diet.

Among the arsonic acids, *p*-chlorophenyl arsonic acid appears most active as a coccidiostat. Arsanilic acid is an effective coccidiostat in the range 0.05 to 0.1 per cent of the diet. Its analogous arsenoxide, arsenoso aniline, is effective at about one-tenth these levels. 3-Nitro-4-hydroxyphenyl arsonic acid is used both as a coccidiostat and a growth promotant in feeds. When used as a coccidiostat, it is recommended at a higher level than for growth alone and is used in combination with a sulfa drug, N-acetyl-4-nitrophenyl sulfanilamide.

The relatively weak in vitro bacteriostatic value of arsonic acids, as compared with antibiotics, is shown in table I.

It is noteworthy that reduction of the pentavalent arsonic acid to the trivalent arsenoso form increases the general bacteriostatic properties greatly as measured by ordinary in vitro tests. Surprisingly, however, chemical reduction appears to destroy the growth-promoting value completely. Little or no correlation is found between the bacteriostatic or coccidiostatic potency of individual arsonic acids and their growth-promoting effects. Shorb et al[7] have shown a stimulatory effect of arsenicals on growth of cecal bacteria from chicks. Thus some of the positive effects of arsenicals may be due to stimulation, rather than suppression of certain of the intestinal microflora.

The greatest value of the arsenicals comes in their general value to improve growth, feed conversion, and resistance to disease, generally as an aid to antibiotics and coccidiostats. Only one arsenical has been used in any one feed. For this reason, arsenicals operate at a disadvantage with regard to coccidiostatic value alone. An arsenical capable of preventing coccidiosis and improving growth and feed efficiency, all at a discreetly safe level, would obviously fulfill a need.

TOLERANCE

Most ingested arsenic is rapidly excreted by animals and is leached from soil, or converted to volatile arsine. The highest concentrations of arsenic are found in

TABLE II

Arsenic Found in Chicken Livers

	Arsenic in feed, ppm As_2O_3	Arsenic in fresh liver, ppm As_2O_3
Arsanilic acid, 0.01%	45.5	1.2
Arsanilic acid, 0.1%	455	6.4
3-Nitro-4-hydroxyphenyl arsonic acid, 0.005%	18.7	2.4
3-Nitro-4-hydroxyphenyl arsonic acid, 0.05%	187	7.5
Dodecylamine *p*-chlorphenylarsonate, 0.01%	23.3	2.9

the liver and kidney. Very little arsenic is found in the bulk of edible tissue, muscle, fat, or skin, even with high-level feeding.

The important question comes with arsenicals as to relative toxicity to host and parasite. The degree of tolerance is clearly related to the amount of arsenic retained by vital tissues. Table II shows the different amounts of arsenic found in chicken livers after long-term feeding of three arsenicals.

The last two compounds are 10 to 20 per cent as well tolerated as arsanilic acid in chickens. The difference is apparently due to differences in metabolism and release of arsenic from the different compounds.

Pigs and turkeys tolerate arsenicals less well than chickens. This again appears related to differences in amount of arsenic retained in vital tissues by the different species. Breed differences as regards tolerance to arsenicals have been observed in both turkeys and swine.

The size of the largest single tolerated dose gives a good indication, in our experience, to the relative toxicity of arsenicals. The data in table III reveal some of these differences.

Tolerance to single oral doses of arsanilic acid and 3-nitro in adult Beltsville White turkeys was 60 and 20 mg./Kg. body weight respectively, reflecting the lesser tolerance to both arsonic acids in this species than in the chick. Daily doses of 2 to 5 mg. 3-nitro per Kg. for six days were tolerated, whereas 10 mg./Kg. caused death in 1 of 3 animals. Arsanilic acid at 10 mg./Kg. per day for six days was tolerated, whereas 40 mg. caused death.

To our knowledge, there has been no authenticated instance of arsanilic acid toxicity in commercially raised birds. This is not surprising, considering the 10

TABLE III

Largest Oral Dose Tolerated in Different Species

	Rat, mg./Kg.	Chicken, mg./Kg.	Duck, mg.
Arsanilic acid	400	300–400	1000
Arsenoso aniline	25	35	
Phenylarsonic acid	10	35	
Dodecylamine *p*-chlorphenylarsonate	< 100	100	
3-Nitro-4-hydroxyphenyl arsonic acid	20	< 100	< 100
4-Nitrophenyl arsonic acid	75	< 100	

to 1 safety margin for arsanilic acid in poultry. We have found, interestingly, that adult turkeys will not continue to consume highly toxic concentrations of arsenicals in feed.

Perhaps the clearest beginning symptom of experimental arsenical toxicity in pigs is stiffness of the hind legs. This may be seen at fairly low concentrations of 3-nitro in feeds. Stiffness of gait has been seen in some pigs raised from weaning on 0.02 per cent arsanilic acid in the feed, twice the maximum recommended feeding level, but not in others.

Improvement in appearance, growth, and feed efficiency is seen even at borderline levels of tolerance for both 3-nitro and arsanilic acid in swine. In the first study conducted with arsanilic acid at 0.01 and 0.02 per cent of a commercial feed, growth increments from weaning to market were 8.3 and 9.8 per cent respectively over the controls. Autopsy revealed no abnormalities in the tissues of pigs fed the 0.01 per cent arsanilic acid level. Some hypercalcification was seen in the bone of pigs fed the 0.02 per cent level for the five and one-half months feeding period. No stiffness of gait was noted in the latter pigs.

Clear evidence of toxicity has not been noted at the 0.01 per cent arsanilic acid feeding level in swine. There has been one suggestion[8] that this might occur, despite a large growth response, in very young pigs on simulated milk diet. On the other hand, studies at Minnesota[9] showed a good growth response with no evidence of toxicity in pigs from three to five weeks on a feed containing 0.0133 per cent arsanilic acid. Hanson, et al[10] fed arsanilic acid at 240 Gm./ton of concentrate free choice with corn, with no evidence of ill effect. The level of arsenic found in the tissues of these pigs was somewhat less than that of pigs receiving 60 Gm. of arsanilic acid per ton in complete feeds.

Dogs tolerate dietary arsanilic acid, showing at least a 100 per cent safety margin above the 0.01 per cent level permitted in complete feeds for livestock. Tolerance to 3-nitro in dogs is close to the 0.005 per cent level. No ill effects were seen in our laboratory throughout reproduction in mink at the 0.02 per cent arsanilic acid feeding level. No ill effects were seen to 0.05 per cent arsanilic acid, as per cent of the dry feed, in mink after whelping.

There appears to be a fair margin of safety for arsanilic acid in calves.[11] Arsanilic acid was less well tolerated than 3-nitro-4-hydroxyphenyl arsonic acid in studies in sheep at Illinois, although both were tolerated at very high levels.[12] There may be a difference between monogastric and ruminating animals in tolerance to different arsenicals. Little or nothing is known regarding the effects of rumen organisms on various arsenicals, or vice versa.

It is noteworthy in considering the role of arsenicals in feeds that they appear to be at least as well tolerated as the various coccidiostats. Problems in their safe use, as with the coccidiostats, come in careful control of their content and distribution in feeds. There is little or no hazard to workers handling arsenicals, with ordinary cleanliness and precautions against dust inhalation.

Hemorrhagic Disease. The original implication that arsonic acids provoke hemorrhage in poultry[13, 14] was not borne out by later studies.[15, 16] The known capacity of sulfaquinoxaline at high levels to cause hypoprothrombinemia and thereby increase need for vitamin K was verified by these studies in the chick. Frost and Spruth[17] critically evaluated the effect of arsanilic acid at 0.05 to 0.08 per cent of the diet on blood clotting time in chicks. Even on a low vitamin K

diet, the presence of 0.05 per cent arsanilic acid did not appear to have a deleterious effect. In the latter studies, sulfaquinoxaline had no demonstrable effect at 0.03 per cent of the diet, well above ordinary recommended level, but increased vitamin K need many fold at the 0.1 per cent level.

Drug induced anemia in chickens has been described recently by Sadek et al[18] with the tacit implication again that arsonic acids may be equally involved with the sulfa drugs. The generalized conclusion is made, "Drugs appeared to be the cause of the condition, either alone or in conjunction with other unknown factors." Such indiscriminate blanket statements regarding "drugs" becloud the real issues. Examination of the observations presented by Sadek et al strongly suggests that one drug alone may well have been responsible for the abnormalities noted.

Combinations of Drugs. Studies in our laboratory have shown no growth inhibition for combinations of 0.04 per cent arsanilic acid in feeds with 0.0125 per cent nicarbazin. Recently Combs and Romoser of Maryland provided us data wherein 0.02 per cent dietary arsanilic acid combined with 0.025 per cent nicarbazin, twice the recommended level of each drug, gave normal growth and feed efficiency in broilers to 9 weeks. Average weight at 9 weeks on this regimen was 3.24 lb., as compared with 3.16 lb. for the group receiving both drugs at recommended levels. Feed efficiency for the two groups was 2.50 and 2.41 respectively. The data by Combs and Romoser also show no aberration from normal for chickens to 9 weeks receiving 0.02 per cent dietary arsanilic acid with 0.0125 per cent nitrofurazone or 7.5 Gm./ton of Furoxone.

Arsanilic acid has been used in combination with all commercial coccidiostats, excepting arsenosobenzene, with no evidence of toxicity. Experience suggests that nitro compounds may interfere with metabolism. Thus, combination of the various nitrated coccidiostats and nitrated arsenicals deserves study on a purely physiologic basis. The combination of certain arsenicals has been shown to work effectively in our laboratory. Where this was done, however, the amount of each arsenical was appropriately reduced. Thus far no such combinations are in use. There is potential hazard in the possible veterinary use of arsenicals at unduly high levels in animals already receiving an arsenical in the feed. This is a common hazard for other drugs, as well. On the other hand, appropriate therapeutic use of arsenicals promises a growing role, particularly in control of scours.

The general philosophy for arsenicals in feeds was expressed previously[2] as follows, "Chemical agents which have a desired activity and good margins of safety have a logical place. The potential hazards involved should be fully recognized and described by the manufacturer of the chemical and by the feed manufacturer who undertakes the responsibility of using the chemical. Once in use, such compounds should remain under constant surveillance by official control organizations and trade associations. The Association of Official Agricultural Chemists is concerned with the development of official methods for the determination of arsonic acids in feeds. These methods provide the best tool for averting trouble at every point."

CONTROL

Feed companies using arsenicals have apparently achieved satisfactory control for several years. The method for arsanilic acid is both accurate and convenient and is now official.[19] Arsanilic acid and sulfaquinoxaline give the same color reac-

tion and must be determined by differential assay. A good method for differential determination of the two drugs in the same feed has been proposed, but is not yet official.

3-Nitro-4-hydroxyphenyl arsonic acid is determined either by a recently devised direct colorimetric procedure, or by determination of total arsenic in the feed, followed by calculation to the equivalence of 3-nitro. The direct method gives higher values, which are thought to more closely represent the true values. 4-Nitrophenyl arsonic acid is reduced chemically to arsanilic acid and is determined conveniently as such.

Arsenosobenzene is determined indirectly by calculation from the total arsenic of the feed. Because the recommended feeding level is low and because the arsenic determination lacks precision, this estimation is subject to a fairly high error. Extra care in premixing is needed where close analytic control cannot be accomplished in the final feed.

The Gutzeit and Cassil-Wichman methods for total arsenic in feeds are official, but they leave much to be desired as to convenience and precision. An improved method for total arsenic deserves attention. The recent Kingsley-Shaffert method and Evans-Bandemer modification[20] may qualify along this line.

VALUE IN FEEDS

Action of arsonic acids closely resembles that of antibiotics. The former are not so generally effective in sparing vitamin requirements. On the other hand, arsanilic acid has been shown to spare thiamin need in chicks, much like penicillin[21] and to decrease N excretion in pigs, the same as chlortetracycline.[22] Gut length and weight in chickens were reduced by penicillin or arsanilic acid.[23] This reminds one of the earlier work at Texas A. & M. in which total clostridia in the droppings of chickens were reduced either by antibiotics or arsanilic acid.[24] Recent work at Iowa State gave a comparable rate of gain in calves for either 3-nitro or antibiotics.[25] Similarly, studies at Minnesota found arsanilic acid comparable to antibiotics in a pre-starter ration for pigs.[9]

Disease Control. One value of the arsenicals comes in controlling certain harmful organisms, not controlled by antibiotics. Cases in point are protection by 4-nitrophenylarsonic acid against histomonads, 3-nitro and arsenosobenzene against coccidia, and arsanilic acid against spirochetes. Part of the extra growth-promoting property of arsonic acids for commercially raised chickens is thought to be due to control of the little understood nonspecific enteritis, or rot-gut.

Although the different arsenicals are effective agents against many diseases, their use must be tempered by the degree of inherent toxicity to the host. In many cases the specific parasites are controlled only by levels bordering on levels that are frankly toxic to the host. Fortunately the arsonic acids, antibiotics, and other drugs complement one another for disease control. Thus the arsenical, bearing only part of the burden, can be strategically used at safe levels.

Combinations of drugs are justified on the basis of efficacy and economics. An example is the use of 90 Gm. arsanilic acid combined with 100 Gm. antibiotic per ton of feed to treat infectious enteritis in swine. Going further, prevention may be accomplished with 90 Gm. arsanilic acid with 50 Gm. or less of antibiotic. Applications to Food and Drug Administration have been made effective thus far

for arsanilic acid with chlortetracycline, oxytetracycline and a mixture of penicillin with streptomycin.[26] The use of 3-nitro at 0.0025 to 0.0075 per cent of the diet in place of 0.005 to 0.01 per cent arsanilic acid qualifies under this regulation. It may be noted also that the regulation applies to prevention and/or treatment of a wide variety of disease states including: CRD, sinusitis, bluecomb, nonspecific infectious enteritis and hexamatiasis in poultry, infectious swine enteritis, and calf scours.

GROWTH, FEED EFFICIENCY, AND TONIC EFFECT

The effect of arsonic acids on appearance, feathering, pigmentation, and haircoat, may be related in part to the little understood tonic effect of arsenic itself. Fundamental study is needed along this line.

Since the pioneering work of Morehouse[27] many investigators have noted improvement in growth and feed conversion due to arsonic acids. As with antibiotics, positive effects have not always been noted. For instance, a progressively diminishing response was noted from year to year in poultry at Wisconsin[21] and at Michigan State[28] for both antibiotics and arsonic acids. Workers at both of these colleges have found, however, that recontamination of their test quarters by droppings from chickens raised under practical growing conditions again induced the antibiotic-arsonic acid response.

Broilers. More than half of the broiler feeds in the country contain an arsonic acid. Feed manufacturers have tested the various arsenicals before adopting them. This fact alone attests to their value. Work to 1954 is reviewed elsewhere.[2] Reference to published work on the arsenicals over the last two years is found in the Proceedings of the A.F.M.A. Nutrition Council.

Turkeys. The following data are illustrative and are shown here because they have not been published previously. These data were reported by J. C. Fritz and E. H. Kramke at the Poultry Science Association meeting in Knoxville in 1951 and are shown here with their permission. Either compound alone appears to stimulate growth slightly. The most significant effect, however, was on feed efficiency when the combination was used (table IV).

TABLE IV

Bronze Poults
(15/group)

Supplement to turkey starter	5 week weight, Gm.	Lb. feed/lb. gain
None	619	3.09
Arsanilic acid, .005%	642	3.00
Arsanilic acid, .0075%	633	2.94
Arsanilic acid, .01%	648	2.84
Arsanilic acid, .0125%	640	2.83
Procaine penicillin, 2 Gm./ton	664	2.99
Procaine penicillin, 5 Gm./ton	662	3.03
Arsanilic acid, .01% + procaine penicillin, 2 Gm./ton	648	2.63

TABLE V

*Data from Study Conducted under Farm Conditions**

Addition to commercial turkey starter	7 weeks, 3 days	
	Av. wt., lb.	Lb. feed/lb. gain
1. None	3.4	2.43
2. Arsanilic acid, 90 Gm./ton	3.6	2.27
3. Procaine penicillin, 2 Gm./ton	3.7	2.41
4. Combination as above (2 & 3)	3.8	2.25

* Two hundred broad-breasted bronze poults/group.

Data of a somewhat similar study, which we conducted under farm conditions, are shown in table V.

These studies clearly show the value of arsanilic acid alone and the greater value when combined with an antibiotic. Pepper and Slinger[29] have reported some value for 3-nitro and arsanilic acid fed to market weight in turkeys; however, the primary value was seen in the poults.

Swine. The value of arsonic acids combined with antibiotics for control of swine dysentery was early shown by Carpenter and Larson.[30] Sodium arsanilate and 3-nitro are widely used in veterinary preparations for control of dysentery. In his report, Patrias[31] summarized the feed use of arsenicals until 1953. In a separate disclosure, Patrias[32] indicated the following as effective treatments for bloody dysentery in swine: arsanilic acid at 0.033 per cent of the feed or 0.015 per cent of the drinking water for five to six days. Alternatively a two day drench providing 25 mg. sodium arsanilate per pound body weight was reported to be effective. Although these treatments are reported to be effective, they are outside the scope of application of feeds to this problem. Recently, as mentioned under the section on disease control, combination of approved levels of arsonic acids with antibiotics have received acceptance for feed use.

R. O. Nesheim conducted a large-scale demonstration of the value of these combinations, providing thereby a basis for Food and Drug Administration acceptance. Such data, shown with Nesheim's permission, on the effects of arsanilic acid in a 16 per cent protein ration in pigs from six weeks to 60 pounds are given in table VI.

TABLE VI

Data on the Effects of Arsanilic Acid with Starter Ration in Pigs

Addition to basal grower ration*	Av. wt. gained/ lb./day	Feed/gain
1. None	.87	3.1
2. Arsanilic acid, 0.01%	.94	2.42
3. Fish solubles and grain fermented solubles	.85	3.31
4. As 3 + arsanilic acid, 0.01%	1	2.56

* Basal ration contains 8 mg. antibiotic/pound. Nine pigs per group from 6 to 10½ weeks of age.

These data show a pronounced effect for arsanilic acid, particularly on feed efficiency. According to Nesheim, improvement in feed efficiency is generally closer to 10 per cent. One may calculate in this regard that it requires an improvement in feed efficiency in excess of only 0.5 per cent to pay for the arsenical in the feed.

Undoubtedly, the greatest response to arsonic acids, antibiotics, or the combination comes when pigs are being stunted by disease. Treatments that improve appetite and general well-being are often critically needed. Just as important, however, the proper diet may be recommended as a preventive during times when outbreaks of dysentery may be expected, such as following weaning, vaccination, cold, damp weather, or moving from one location to another. On farms where dysentery is endemic, periodic feeding of an arsonic acid-antibiotic diet for three to five days each two to four weeks may be advised. Intermittent feeding of such therapeutic diets proves economical in situations in which mortality or morbidity are significantly reduced.

ARSONIC ACIDS IN EGG PRODUCTION

An interesting facet of study with arsonic acids is their possible role in egg production. In his early study Morehouse[27] presented evidence that pullets receiving 3-nitro commenced laying about 15 days earlier than birds not receiving this compound. At 24 weeks of age, the mean weight of the pullets receiving 3-nitro was greater than that of the controls, indicating earlier maturity. There was no difference in weights of eggs from the 3-nitro and control pullets. Morehouse[33] describes various studies confirming the original one. Pepper, et al[34] in a preliminary report noted a trend toward earlier sexual maturity in Columbian pullets receiving 3-nitro. Although experiments are continuing in separate areas, Morehouse concludes that the data accumulated thus far indicate that (1) pullets reach earlier physical and sexual maturity, (2) hens show greater livability, (3) hens lay more eggs, (4) hens convert feed into eggs more significantly, and (5) fertility and hatchability is not significantly affected.

The safety of arsanilic acid for egg production was adequately established by Libby, et al[35] wherein arsanilic acid was fed at 0.01 and 0.02 per cent of the diet with no ill effects on hatchability or egg production. Evans et al[36] and Evans and Bandemer[20] found insignificant levels of arsenic in the eggs or in the tissues of hens fed arsanilic acid continuously at the 0.01 to 0.02 per cent feeding levels. Carlson et al[37] reported improvement in egg production and feed conversion in pullets receiving arsanilic acid at 120 Gm./ton on a 12 per cent protein ration. Creech et al[38] at Texas A. & M. found egg production increased in heavy breeds equally by 0.01 per cent arsanilic acid or by antibiotics. The latter experiments were conducted in New Hampshire pullets over a five month feeding period. The drugs were introduced in the Texas study after the pullets were already in production.

In a recent study by Libby et al,[39] arsanilic acid, 0.01 per cent, in White Rocks from day old gave 38.4 per cent egg production versus 33.2 per cent for controls over the first egg production year. The arsanilic acid hens used 8.2 pounds of feed per dozen eggs versus 8.5 pounds for the controls. Age at first egg was seven days less for the arsanilic acid group.

TABLE VII

The Effect of Arsanilic Acid on Selenium Toxicity

Addition to basal ration	Av. initial wt., lb.	Av. final wt., lb.	Selenium toxicity, no. of pigs*
None	26.4	156.2	0
Se, 10 ppm	26.2	137.2	2
Se, 10 ppm + arsanilic acid, 0.01%	26.1	154.8	0
Se, 10 ppm + chlortetracycline, 5 mg/lb.	26.4	166.2	4
Se, + arsanilic acid + chlortetracycline	26.4	170.5	0

* Eight pigs per group started.

The possible role of arsenic in reproduction has been reviewed in our earlier reports. We investigated the effect of 0.01 and 0.02 per cent arsanilic acid in our regular breeder ration on the reproductive performance of rats over three generations and three matings of the first generation. There was clearly no interference with litter size, weight, or survival to 21 days due to the arsonic acid. If anything, there was an increase in the average number per litter for rats which received the arsanilic acid. These findings appear to warrant thorough investigation of arsonic acids on reproductive performance.

COUNTERACTION OF SELENIUM TOXICITY

One of the most intriguing possibilities for the arsonic acids in feeds is counteraction of selenium toxicity in those areas in which seleniferous grains represent a real problem. The possibility exists that organic arsenicals will prove superior to sodium arsenite for control of selenium poisoning, or alkali disease, as it is sometimes called. Background for study along this line is found in the earlier publications of Moxon et al[40] and Moxon and Wilson.[41]

The value of arsanilic acid and 3-nitro against selenium has already been shown in rats by Hendrick et al,[42] in chickens by Carlson et al,[43] and in pigs by Wahlstrom et al.[44] Studies with arsanilic acid to counteract selenium toxicity in steers are now being made by Olson and others at South Dakota. Background for this work is found in a paper by Moxon et al.[45]

The effect of arsanilic acid in presence of chlortetracycline on growth of pigs on seleniferous diets is shown in table VII, as presented by Wahlstrom at the 1955 Animal Production Society meeting.[46] They are shown here with his permission.

The combination, arsanilic acid with chlortetracycline, appeared to protect the pigs against the selenium and at the same time allowed better growth than the controls not receiving selenium. As reported by Wahlstrom et al, the arsonic acids alone do not provide the complete answer to the selenium problem. It is possible, however, that they can be used to advantage with other materials that also help counteract selenium toxicity.

SUMMARY

The contemporary position regarding organic arsenicals in feeds is reviewed. Four compounds are now in use as aids to animal production. Certain arsenicals have more or less specific value for control of coccidiosis, blackhead, swine enteritis, and other diseases, and for growth promotion and improvement in feed efficiency. No clear relation has emerged between structure and activity.

The various arsenicals differ markedly, one from the other, in toxicity. There are also wide differences in tolerance to arsenicals between different animal species. These differences are reflected in the amount of arsenic found in the livers of animals fed the arsenical. From the evidence at hand, there appears to be no real basis to relate the recent occurrence of the "hemorrhagic syndrome" of poultry to arsenicals. On the other hand, arsenicals, like all other useful drugs, have some measure of toxicity when used at high enough levels. These measures of toxicity have been reviewed.

Knowledge of proper limitations and conformance to existing regulations will help insure continued safe use of arsenicals. Millions of pounds of arsenicals have been used in feeds in the last five years. As now used, the arsenicals do not appear in any way to represent a hazard to human health.

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Feed Control and Special Assay Problems for Diethylstilbestrol

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Feed control has its beginning in the very early years of our western hemisphere, dating even to the time before the Revolutionary War. As different grains were mixed, physical inspection, especially if made of the ground material, could not be relied upon to judge the quality, and so some technical control became necessary to protect both the seller and the buyer.

Progress in feed control was slow and varied from state to state. It was evident that similar questions existed in many states. About 1910, a group of feed control officials met with members of the American Feed Manufacturers Association, and, at the conclusion of these discussions, the present Association of American Feed Control Officials was born. Steady continued growth has produced a strong group of individuals whose regulatory principles are accepted and understood nationally. Such development is logical and proper on a firm basis and provides a set of operating definitions and methods of test that are the backbone of the industry. The expansion of the feed industry, particularly as to the addition of antibiotics and vitamins, has presented a very large and complex problem to the feed control officials. Their efforts to develop definitions and methods of assay are significant and the revisions that appear are published annually.

The American agricultural scientist's continuous search for more efficient methods of producing food, especially meat, has accelerated the production of medicated feeds and helped to expand our 3.5 billion dollar commercial feed manufacturing industry.

Today, the farmer can literally feed away many diseases and prevent still more from occurring in his herds or flocks by the simple addition of a few grams of one of our wonder drugs to a ton of livestock feed.

As the demand for more and more quantities of feed increased the need for larger capacity, blending apparatus developed and, as may be expected, bigger and better equipment was designed, manufactured, and installed at plants of feed manufacturers. There does not appear to be any limit to the amount of feed that can be blended per hour. A well-designed feed milling and blending operation depends on having available adequate railroad siding capacity, warehouse storage, and bagging machines to take care of the production properly from the actual blending apparatus.

The incorporation of such minute amounts of drugs in the feed carrier presents a number of problems. It is necessary that the drug and feed mixture be made as homogenous as possible without such an excessive amount of mixing as to make

the operation uneconomical. In general, the finest possible particle size should be used. If a large particle size must be used, then it is necessary to provide a very thorough mixing in order to assure proper distribution of the particles.

Today a highly competitive spirit exists in the feed industry as it does throughout much of American business. The desire and need to be first on the market with a new product places increased pressure on the nutritionist and analyst. Complete and accurate work must be turned out in a limited amount of time; therefore, problems of stability, compatability, and methods of testing become very challenging and demanding in order to meet the requirements of modern business without sacrificing quality and accuracy.

The analytic control of new products is by no means simple, but it is extremely important. Some of the control points have been stated, and there is one further point that concerns interference of the various added medicaments on the assay process of the specific additive under test. Time and experimental runs are required to prove the usefulness of any control procedure for new feeds.

The absolute need for feed control is universally accepted. All feeds and feed ingredients should be sold on a guaranteed analysis for the protection of the feed manufacturer as well as the user. It would require too much time here to discuss all the problems confronting the control chemist. We are all aware of the workings of The Association of American Feed Control Officials and the Association of Official Agricultural Chemists as well as the many other similar groups, which are working to develop satisfactory control procedures that assist in solving the problems concerned with detection of various additives in feed.

It may perhaps be wise to list some of the basic additives used in feeds, and these include arsenicals, coccidiostats, vitamin B_{12}, antibiotics, vitamins A and D, and diethylstilbestrol.

We do not intend to go into the separate methods of assay for each or any of these ingredients except diethylstilbestrol, but we are sure that those who have had experience with analytic procedures are aware of the complications that arise when such feed supplements are submitted to test.

Before describing the assay procedure, it may be well to mention something about the hazards that exist with the handling of medicated feeds.

The usual precautions should be exerted as would be expected to be done with pharmaceutic materials used for humans. Empty containers should be destroyed by burning, and partially used packages should be placed in suitable spots where they are unavailable for other animals or humans, especially young children.

MATERIALS AND METHODS

Numerous methods for the determination of diethylstilbestrol are reported in the literature. Most of these methods of analysis depend on reactions involving the phenolic group. Some of the methods are: the irradiation method, the absorptionmetric method, Folin's method, the nitroso method, the bromination method, and the antimony pentachloride method.

Our experience indicated that some variation of the irradiation method originally introduced by Eli Lilly & Co. is more accurate and easier to carry out on low level feed samples than any of the other methods. The nitroso, Folin, and bromination methods are better for larger amounts of diethylstilbestrol. The antimony

pentachloride method can introduce troubles due to excess color of the extract and is not as easy to complete as the irradiation method.

The following is an outline of the method used in our laboratories for the determination of diethylstilbestrol in feeds.

Reagents and Apparatus. The following materials are used: 2 per cent sodium hydroxide solution; glacial acetic acid, reagent grade; chloroform, reagent grade; ultraviolet lamp; Beckman spectrophotometer, tungsten lamp; matched 1 cm. clear quartz cells; U.S.P. diethylstilbestrol; prepared Florisil column, diameter 1.6 cm., length 11.4 cm. The Florisil should be 60 to 100 mesh, suspended in chloroform, and transferred to the column. Use 6 Gm. of Florisil, and tap to the proper height.

Preparation of the Sample. In the case of most feeds, the sample should be mixed, quartered, and used for analysis. In the case of pelleted feed, the sample should be ground. If the molasses content is high, the sample should be ground with an equal weight of sand.

Procedure. Transfer a 20 to 25 Gm. sample to an extraction thimble. Extract the sample in four hours, using a Soxhlet apparatus and 150 ml. of chloroform as an extraction solvent. Cool the extract, leaving part of the chloroform in the upper part of the apparatus so that the measured extract volume is a little less than 100 ml. Pass 50 ml. of this extract through a prepared Florisil column, then wash diethylstilbestrol through the column using 150 ml. of water-saturated chloroform. The rate of flow should be reasonably rapid, about three-quarters of an hour. Transfer the eluate and washings to a 300 ml. separatory funnel. Extract it with two portions of 2 per cent sodium hydroxide solution (40 and 10 ml.). If an emulsion forms, break it up, using a centrifuge. Wash the chloroform layer with 10 ml. of water. Finally, combine the alkaline extractions plus the wash water and wash with two 20 ml. portions of chloroform. Rinse this wash chloroform with 5 ml. of water. Adjust the *p*H with 2 *N* phosphoric acid to a *p*H of 8.0 to 5.0, depending on the type sample. Extract with chloroform using two portions (40 and 10 ml.). Wash the combined chloroform extractions with 5 ml. of water. To the chloroform add 1 Gm. of anhydrous sodium sulfate. Mix and filter through a small plug of glass wool. Wash the residual sodium sulfate with two 5 ml. portions of chloroform. Measure the final volume of chloroform. Transfer 15 ml. portions into two small beakers. Evaporate off the chloroform, using an air current. Take up the residue in exactly 10 ml. of pure glacial acetic acid. Mix and transfer to standard 1 cm. clear quartz cells. Determine the transmission, using a Beckman spectrophotometer at 420 mμ. Irradiate for six minutes at a distance of six inches from a standard ultraviolet lamp. Determine the transmission of the solution. From a standard graph, determine the amount of diethylstilbestrol present in the aliquot used. Apply the necessary correction factor and then calculate the diethylstilbestrol in the original feed sample.

Preparation of a Standard Graph. Dissolve 25 mg. of U.S.P. diethylstilbestrol in 250 ml. of chloroform. Dilute this solution 10 ml. to 100 ml. in a volumetric flask with chloroform. Evaporate aliquots of this dilution containing 30, 50, and 100 γ of diethylstilbestrol. Dissolve these residues in 10 ml. of glacial acetic acid. Irradiate these solutions under standard conditions. Determine the transmission at 420 mμ and plot the results on semilogarithm paper.

Standard Conditions of Irradiations. Place an acetic acid solution containing 50 γ of diethylstilbestrol in 10 ml. in a 1 cm. clear quartz cell at a distance of six

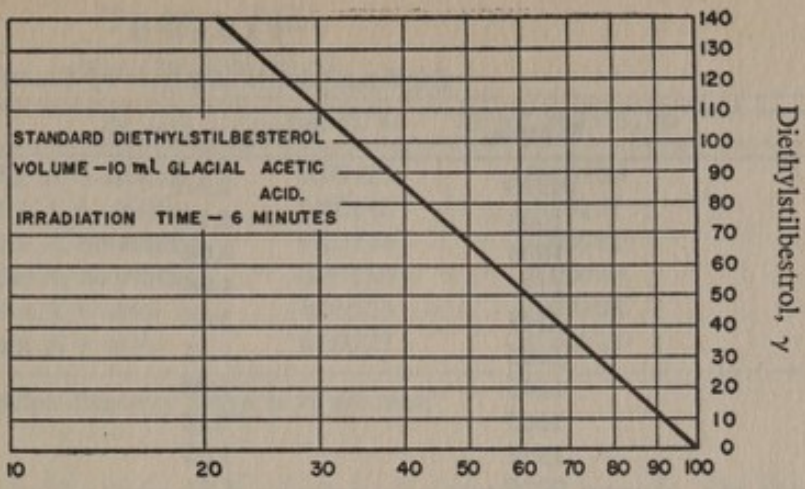

FIGURE 1.

inches from the ultraviolet lamp. Irradiate, removing the sample at one minute intervals and determining the transmission at 420 mμ. Plot the transmission readings against time and determine the time of maximum color intensity. Use this time for irradiation in preparing the standard curve and for the analysis of feed samples (fig. 1).

Method of Correcting for Physical and Chemical Losses. In both methods use a standard premix sample for added increments. In the recovery factor method, weigh out a 25 Gm. sample of a blank feed in a thimble, as usual. Add a weighed amount of diethylstilbestrol premix so that the amount in the final extract is 300 γ diethylstilbestrol. Carry through the analysis as previously described and calculate the per cent recovery of the added diethylstilbestrol. On the basis of this recovery factor, calculate the diethylstilbestrol in the feed sample.

In the increment method, to a 25 Gm. sample of the feed weighed out in the thimble and placed in the apparatus, add either a weighed amount of premix equal to 200 γ of diethylstilbestrol or a chloroform extract of a premix containing the same amount of diethylstilbestrol. Proceed with the analysis as usual. Calculate the recovery factor of the added increments from the results of the analysis of the feed alone and the feed plus the added increment. Use this factor to determine the correct amount of diethylstilbestrol present in the feed sample.

The following notes give a few variations and explanation of some of the steps in the method.

1. In the case of feeds low in alfalfa, the length of the column can be cut in half. Those samples with high alfalfa use a six inch column. With feeds having no emulsifying components and low in color, the column step can be omitted.

2. In the case of feeds in the form of hard pellets, they should be ground and extracted for 8 to 12 hours. Feeds containing a high molasses content should be ground with an equal weight of sand and extracted at least eight hours.

3. The alkaline extraction should be carried out with very gentle shaking; emulsion should be avoided. In some cases, 5 per cent urea should be added to complex some of the emulsifying components. The separation of the aqueous and chloroform layers should be complete, and the aqueous fraction should be washed twice with chloroform. The object of this technique is to obtain a low blank in the final acetic acid solution.

TABLE I

Diethystilbestrol Analysis of Premixes

Sample	Brooklyn, Gm./lb.	Vigo, Gm./lb.
0995	1.17	1.18
1005	1.10	1.09
1095	1.08	1.11
1135	1.06	1.07
1545	1.06	1.09
1605	1.13	1.10
1645	1.08	1.02
1665	1.12	1.10
1995	1.06	1.08
2005	1.11	1.08
2035	1.11	1.09
Average	1.098	1.091

4. In the case of feeds containing oxytetracycline (Terramycin*), it is better to adjust the *p*H to 5.

5. The final residue should be assayed as soon as it is dry.

6. The aliquots used in this analysis are for feeds containing 5 mg. of diethylstilbestrol per pound. In the case of feeds containing larger amounts of diethylstilbestrol per pound, one should use a smaller aliquot for evaporation in the final step. The best range of diethylstilbestrol for irradiation is between 20 to 50 γ.

In the case of feeds containing 1 mg. of diethylstilbestrol per pound, it is necessary to use a 100 Gm. sample, a large extraction apparatus, and a number of other special steps depending on the other components present.

Table I gives the analytic data on a number of premix concentrates containing 1.1 Gm. of diethylstilbestrol per pound. The analyses indicate the analytic work of two laboratories. Considering the nature of the samples, the results are satisfactory.

Table II gives the analysis of a number of actual feed samples from the trade, analyzed by the irradiation method, and indicates the actual recovery of diethylstilbestrol and also the increment factor found by the recovery of a known added

TABLE II

Diethylstilbestrol in Feeds

Sample	Estimated potency, %	Uncorrected potency, %	Recovery factor, %	Corrected potency, %
A	0.0011	0.00116	98	0.00118
B	0.0022	0.00196	90	0.00218
C	0.0022	0.00216	95	0.00227
D	0.0011	0.00102	92	0.00111
E	0.0011	0.00103	90	0.00114
F	0.0022	0.00235	98	0.00240
G	0.0022	0.00205	95	0.00216
H	0.0011	0.00105	92	0.00114
I	0.0022	0.00207	95	0.00218
J	0.0011	0.00114	95	0.00120

* The trade name of Chas. Pfizer & Co. for oxytetracycline is Terramycin.

TABLE III

Vigo Stability Study

Sample no.		Initial, %	12 wk. at 45 C., %
F-0118B	Purdue A + alfalfa	0.00127	0.00120
F-0118C*	Purdue A + alfalfa	0.00118	0.00117
F-0118E	Purdue A, no alfalfa	0.00114	0.00081
F-0118F*	Purdue A, no alfalfa	0.00110	0.00073
F-0118H	Purdue A + urea	0.00123	0.00108
F-0118I*	Purdue A + urea	0.00123	0.00124

* Contains oxytetracycline. Recovery factor is 95 per cent.

amount of diethylstilbestrol in the form of a solution of a standard premix. On the basis of this factor, the actual diethylstilbestrol present in the feed is calculated. The recovery factor varied from 90 to 98 per cent, depending on the type feed.

Table III gives the analysis of a number of synthetic samples as made and held at room temperature and also held at 45 C. for 12 weeks.

CONCLUSION

We have tried briefly to give some of the problems that the analytical control chemist meets in the feed industry. Finally we have attempted to describe as elaborately as possible the process that we are using in assaying feeds to which diethylstilbestrol has been added. The most appropriate reward that we can expect is that this résumé has alerted all to the complexity of the problems and to a better understanding and appreciation of the analyst's position in this important and growing feed industry.

ACKNOWLEDGMENT

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Public Health Significance of Drugs in Animal Feeds

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What could be called a minor revolution in the animal feed industry in this country is the very rapid increase in the use of "medicated feeds." Medicated feeds are those that contain various hormones or drugs for medicinal or growth-promoting purposes for cattle, poultry, swine, and other forms of livestock. At the present time it may be estimated that approximately 7 to 9 million tons of medicated feeds are manufactured in this country each year, which is about 20 to 25 per cent of all manufactured feeds.

Although the farmer has administered medicines to his animals in their feed for many years (usually on the advice of his veterinarian and often with the help of a local feed mill), only since the Second World War have medicated feeds been used widely on a national scale. Briefly, their increased popularity is due to several factors including: (1) the present availability of low cost, stable, and efficient drugs for specific diseases and of effective hormone-like materials; (2) the ease, effectiveness, and safety of administering drugs in the feed to large numbers of animals on a daily basis (some poultry flocks, for instance, number up to 50,000 birds per house); (3) the remarkable growth of the modern feed industry.

Examples of drugs now routinely given to farm animals in the feed for various purposes include at least five different antibiotics (bacitracin, streptomycin, chlortetracycline, penicillin, and oxytetracycline), several organic arsenicals, plus sulfaquinoxaline, nicarbazin, nitrophenide, nitrofurazone, furazolidone, and 2-amino-5-nitrothiazole. Several of these are used as growth-promotants, and others are used for the control or partial control of such diseases as scours in calves; baby pig diarrhea, dysentery, and necrotic enteritis in swine; and coccidiosis, hexamitiasis, respiratory disease, blue comb, infectious sinusitis, and blackhead in poultry. In addition, several hormone-like substances, such as stilbestrol or dienestrol diacetate, are used in beef cattle and poultry feeding. Also, several anthelmintics, such as phenothiazine, are used to control *Haemonchus, Ostertagia,* and *Trichostrongylus* in cattle.

PUBLIC HEALTH SIGNIFICANCE

From the public health viewpoint, there are two questions that might be asked concerning the use of medicated feeds. First, what possible harm to the health of mankind might result from the use of such feeds and, second, what benefits to public health may occur?

In answer to the first question, possible harm from the use of medicated feeds could theoretically result from a "carry-over" of drug residue in meat, milk, or eggs with eventual consumption by man. However, it is fortunate that through the careful vigilance of the Food and Drug Administration, in cooperation with drug manufacturers, the feed-manufacturing industry, colleges, research laboratories, and feed control officials, no drugs are used in medicated feeds that have not been proved to be safe.[1] However, continued caution and study are necessary, since, over long periods of time, drug residues in food could conceivably cause harm, as outlined in the following sections.

Possible Toxic Reactions from the Accumulation of Drugs in the Body. Many of the drugs in present use have little or no carry-over into food and, hence, there is no public health problem with these drugs in most instances. Where such carry-over might occur, it is possible to remove the drug from the animal's feed for a period of time before slaughtering, which results in the disappearance of the drug from the food. In some cases, such as with some of the antibiotics, the drug that might be present in the food is destroyed by cooking or has been shown to be otherwise harmless.

In this connection, the safety of poultry meat dipped in chlortetracycline, for the purpose of increasing the shelf life of fresh meat, has been recently demonstrated, and this procedure has been approved by the Food and Drug Administration for commercial use. It may become commonplace in the future for man to consume meat treated with certain antibiotics. In fact, it is likely that man has consumed small amounts of antibiotics in his food from the beginning of time in various fermented products and foods or drinks of microbiologic origin. Also, it is known that since the advent of the use of penicillin for mastitis in cattle, as much as 11.6 per cent of the milk available in our larger cities contains up to 80 units of penicillin per quart.[2] The authors stated that "much of this would be inactivated by action of the intestinal flora and little absorbed" and did not warn against the consumption of such milk except by extremely sensitive persons.

Meat from hormone-treated animals presents a similar problem, and continued caution is necessary. However, again, what residues remain in the food, if any, are too small to be of concern under present conditions, in spite of certain dire claims in the popular press. It has been estimated that one would have to consume at least 22 lb. of beef in one day to get enough stilbestrol to have an effect on the body.[3] The problem of residues in animal tissues is discussed elsewhere on this program in more detail.

Possible Development of Resistant Strains of Pathogenic Organisms. No important resistant strains of organisms infectious to man have developed in animals or human beings from the use of drugs in animal feeds as far as is known. It is well known, however, that resistant strains of organisms do result from the intake of therapeutic amounts of antibiotics by man,[4] so continued vigilance by all those concerned in the manufacture and control of medicated feeds is necessary.

Development of Sensitivity in the Consumer. It is known that a small percentage of our population is sensitive to penicillin and certain other antibiotics. However, as far as we are aware, no reaction has occurred as a result of consuming food obtained from animals receiving medicated feeds. Fortunately, there is much less chance of becoming sensitive to antibiotics taken orally than to injected antibiotics. Rather than stop this entire program of medicated feeds, if such sensi-

tivity is found in relatively few individuals, it would seem more sensible to make available meat, milk, and eggs from animals fed nonmedicated feeds for the exceptional individual (just as low sodium foods are now available).

Medicated Feeds Not a "Cure-All." Medicated feeds should not be considered a "cure-all" for the control of animal diseases. Only a relatively few animal diseases are controlled by drugs in the feed. Hence, it is as important as ever that farmers follow the usual sanitary procedures and use other disease control methods as necessary.

Benefits from Medicated Feeds. As far as benefits to mankind from the use of medicated feeds are concerned, the main direct benefit is the present availability of more and better foods at relatively less cost to the producer and to the consumer. The use of medicated feeds has resulted in up to 20 per cent increase in growth rates, improved feed utilization, and lowered mortality. The entire food-producing potential of the country is increased, as discussed elsewhere in this program. This will be especially important in periods when food is in short supply due to poor weather conditions or in other periods of national emergency.

The farmer benefits in a direct manner by the control of certain animal diseases. In this connection, the public may benefit in the future by the possible control of certain animal diseases transmissible to man. Such studies are in the experimental stage at present but have great promise for the future.

CONCLUSIONS

We can conclude that with the ever-present, but important, cautious control by the Food and Drug Administration, in cooperation with state officials and the feed and chemical industries, the benefits of medicated feeds far outweigh any evidence of possible danger to mankind from their use. It is likely that the use of medicated feeds is only in its infancy and that as far as the public health aspects are concerned, the future looks bright as long as continued vigilance is given.

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Medication under Conditions of Stress

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Stress has been defined[1] as a "non-specific deviation from the normal resting stage," and as a "reaction to stimulus." It possesses nonspecific features of disease, but no "eliciting pathogen" is isolated. The stress phenomenon follows a fairly consistent pattern, which is termed "the general adaptation syndrome."

Stress is not a disease, but is inherent in life itself. True, it is caused by an "alarming stimulus," and its course may be greatly modified by secondary or superimposed presence of a recognized pathogen, but stress is the result, not the cause. Numerous factors of stress have been listed.[2, 3] Among the major ones for poultry and livestock are: abnormal environmental conditions, major surgical procedures, drugs, vaccination and other trauma, extreme physical exertion, toxins, and fever. In almost every instance, reduced feed consumption is an early manifestation, and this adds undernutrition and malnutrition as another complication.

In livestock and poultry farming, the impaired nutrition is doubly important, because it is readily recognized and can be quickly corrected.

PHYSIOLOGIC CHANGES

The stress phenomenon or the general adaptation syndrome occurs in three distinct phases: (1) the alarm reaction, (2) the stage of resistance, and (3) the stage of exhaustion.

The first changes in the alarm reaction are noted in the endocrine glands, and specifically as activation of the pituitary-adrenal system. This means increased release and utilization of the hormones, especially those of the adrenal cortex.[4] This endocrine activity creates in the body a significantly greater need for essential nutrients—some for the repair incident to accelerated cell metabolism and some for detoxifying the waste products of this process.

The net result is that body requirements are above and beyond the usual or average needs. This is coupled with reduced nutrient intake, and a nutritional deficiency develops on a ration that had been adequate for the needs under normal or usual conditions. This deficiency is almost invariably multiple under field conditions, but it may be single and specific under experimental conditions. It would be erroneous to say that stress increases the nutritional requirements, because stress is the result of a physiologic attempt to maintain a normal tissue balance (homeostasis) under unfavorable conditions. What actually happens is that the

alarming stimuli set up a reaction that creates a need for larger quantities of specific nutrients.

The second phase, the stage of resistance, is the true objective of the body, because it indicates that the body has made all adjustments necessary to adapt to the change and has, in effect, accepted the alarming stimuli as normal.

The third phase, the stage of exhaustion, is reached only in failure, since it indicates inability to adjust to and live under the changed circumstances.

MEDICATED NUTRITION

Diet and the nutritive state are intimately related to adaptation to stress, and nutritional factors are important in all three phases of the syndrome, as they are in all normal physiology and growth. Nutritional deficiency impairs resistance to stressor agents or alarming stimuli.[5] This may occur because chronic undernutrition is, of itself, a nonspecific stress resulting from low caloric intake or because excessive adrenal activity depletes this organ of some of the vitamins, amino acids, and other substances essential for the synthesis of the hormones.

Undoubtedly, the two factors are inseparably involved, for the adaptation can be achieved either by supplying added amounts of the adrenal hormones or by supplying added amounts of the limiting vitamins and amino acids, so that the adrenals can again fill the need for the increased hormones demanded by the organs and tissues of the body. Prolonged restriction of caloric intake itself depresses the formation and release of a number of anterior pituitary hormones and those of their target organs.

The relationships between the nutritive state and the adaptation syndrome are at times specific, at other times nonspecific; they may be direct or indirect; and they may be the result of deficiency or excess, and may be traceable to diet or other causes.[4] Nutritional status may affect the balance by altering the synthesis and secretion of hormones, by changing the response of the target organs and tissues, and by influencing the metabolism and excretion of hormones. At the same time, the endocrines may alter the absorption, utilization, and excretion of nutrients, or they may modify the body requirements for specific factors.

The added nutritive substances that serve to restore normal physiologic function to the pituitary-adrenal system, and to the entire body, may be considered under antibiotics, vitamins, amino acids, minerals, and unidentified factors. It is believed[6] that adaptation occurs at the cellular level, that it involves the enzyme systems in the cells, and that the malnutrition may be caused, in part, by factors other than an incomplete or insufficient ration—interference with absorption or with utilization, increased requirement, excessive destruction, and rapid excretion.

ANTIBIOTICS

The use of antibiotics at low levels for growth promotion is widely accepted, and their use at high levels for medication, and particularly during periods of stress, is gaining in popularity. The latter procedure is logical, since one of the primary functions served in periods of stress is to improve the appetite and encourage consumption of greater quantities of feed. Other effects may be accounted for by

a sparing action on vitamin B, by improved digestibility of nitrogen, and by decreased excretion of endogenous nitrogen.

Improve Appetite. As feed consumption approaches normal, the body again has available those nutrients that are needed—assuming that the ration has been formulated to provide all nutrients in proper amount and proportion for maximum performance (growth, production, reproduction).

Two reasons have been suggested[7] to account for the improved feed consumption: the increased numbers of coliform bacteria help to stimulate appetite or the decreased numbers of enterococci remove appetite-inhibiting factors. To this latter group, the toxin formers—the clostridia and other anaerobes—may be added.

The coliform organisms synthesize several fractions of the vitamin B complex[8] —folic acid, riboflavin, niacin, and B_{12}—and these are produced as extracellular products that are available to the bird. Chicks fed procaine penicillin and pure cultures of certain strains of *Escherichia coli* and *Aerobacter aerogenes* increased the rate of gain by 64 to 80 per cent over controls without penicillin.[9] Since these organisms are known to be resistant to penicillin and bacitracin, but sensitive to chlortetracycline, oxytetracycline, and streptomycin, the choice of antibiotic will affect the coliform counts. It has been suggested also that "a balance of types of organisms" must be maintained[10] in order to achieve a favorable growth response in chicks, and the favorable balance, as shown by fastest growth, is a reduced number of anaerobic rods plus an increased number of coliforms. Bacitracin, penicillin, or a combination of the two will permit normal growth of the coliform bacteria.

Enterococci and specifically *Micrococcus pyogenes* produce toxins that cause birds to develop enterocolitis, fever, anorexia, and diarrhea.[11] Clostridia also produce toxins [12] and have been mentioned as a cause of depressed appetite and poor growth. Adding penicillin and/or oxytetracycline to drinking water for turkeys reduced the *Clostridium perfringens* count while improving rate of growth.[13] The authors suggest that the reduced number of clostridia resulted in less toxin synthesis and hence less depression of appetite and growth.

Sparing Action. Stress produces reactions in the body that increase the demands for vitamin B while at the same time reducing the supply by depressing the appetite.[14] It has been shown that addition to the ration of penicillin, chlortetracycline, and streptomycin greatly improved growth when thiamine was present in marginal amount (0.5 mg./Kg. of ration).[15] In this trial, rats on the control ration weighed 55 Gm. after four weeks, those on penicillin 115 Gm., on chlortetracycline 76 Gm., and on streptomycin 69 Gm. Oxytetracycline and chloramphenicol gave no response in this test.

Both penicillin and chlortetracycline improved growth in rats being fed rations containing marginal amounts of riboflavin, as well as those low in pantothenic acid.

Antibiotics also improve nitrogen utilization when the rations being fed contain minimal amounts of methionine.[16] This test showed that equal response was secured with added antibiotic (streptomycin, chloramphenicol) or added methionine, while adding both produced a response closely approximating the sum of their individual effects. In these tests, the addition of antibiotic increased the true digestibility of nitrogen consistently, but slightly, and reduced the excretion of endogenous nitrogen. This seemed to indicate that, of the nitrogen absorbed, less was needed to maintain the integrity of the nitrogen-containing tissues of the body.

In stress, this would mean a longer period of increased demand before the supply of nitrogen was depleted, hence a longer period for adaptation before exhaustion.

Whether this sparing action is direct has not been established, but it seems probable that the antibiotic may help to conserve the nutrients for the host by also reducing competition from bacteria in the digestive tract and by controlling the secondary invaders that might otherwise penetrate the tissues and exert still further stressor forces.

Reduce Competitors. Bacteria that compete with the host for preformed nutrients supplied by the ration or elaborated in the gut serve as stressor stimuli, when these nutrients do not become available through digestive actions later in the process. Antibiotics can reduce the stress by checking the competing bacteria and helping to maintain a balance between the several types of organisms present.

An unusual increase in numbers of *Lactobacillus bifidus* was correlated with poor chick growth,[17] and it was postulated that these organisms were competing with the birds for mutually essential nutrients. When penicillin was added to the ration, the number of bifids was reduced and growth improved.

Control Pathogens. Pathogenic organisms, those capable of producing disease, may be the inciting or primary stressor stimuli, but more frequently are opportunists or secondary agents. Antibiotics can prevent or reduce the severity of damage caused by stress of this type. Results are highly variable, however, because the outstanding characteristic of the antibiotics, singly and as a group, is the high degree of selectivity shown as regards the species and even the strains and families of bacteria that may be inhibited or killed. Bacitracin and penicillin are highly effective against such common opportunists as streptococci, staphylococci, and clostridia. Moreover, it is generally agreed that the antibiotics act while they are in the digestive tract,[18, 19] and most investigators report that systemic infections are not adequately treated by oral administration of antibiotics.

The value of antibiotics in medicated feeds for use during periods of stress lies chiefly in their ability to stimulate appetite, to prevent excessive multiplication of pathogenic and competitive bacteria, and to maintain a normal balance among the many types of bacteria always present in the digestive tract. Bacitracin and penicillin encourage a balance between synthesizers and pathogens.

VITAMINS

Vitamins to be considered are chiefly, but not exclusively, water-soluble: pantothenic acid, riboflavin, thiamine, choline, pyridoxine, vitamin K, ascorbic acid, and vitamin A.

Pantothenic acid affects both the structure and the function of the adrenal cortex,[20] and it is critically important in maintaining the integrity of the cortical cells. The requirements of the body depend on its physiologic state. During periods of stress, the demand for pantothenic acid is increased, and the supply is depleted in proportion to the severity of the stress. Deficiency results in reduced feed consumption, atrophy and necrosis of the adrenal gland, and sometimes hemorrhage in the cortex. Deficiency also affects the response of lymphocytes and eosinophils, the synthesis of antibodies, the metabolism of carbohydrates and fats, the production of adrenal steroids from the fats, and the capacity of rats to swim

in cold water. If deficiency is prolonged, adrenal cortical exhaustion supervenes and death ensues.

Large doses of pantothenic acid help condition the response to stress, in depleted animals as well as those eating normal rations.

Riboflavin is required for the synthesis of adrenal hormones, or in the mechanism of their elaboration.[21] The action may be direct. A deficiency impairs pituitary-adrenal function in the rat, interfering with either the synthesis or the secretion of ACTH. Deficiency also prevents the normal increase of liver glycogen levels in animals held under low oxygen tension. Injected riboflavin corrects this inability, as does administration of cortin. Doubling the riboflavin content of the diet corrected the deficiency, and it was observed that both penicillin and chlortetracycline were just as effective as doubling the riboflavin.[14]

Thiamine is a potent activator of the pituitary-adrenal system of the rat, mouse, rabbit, dog, monkey, and man. Deficiency causes changes that are characteristic of nonspecific stress and that follow the pattern of the general adaptation syndrome.[2] Reduced feed consumption and lowered caloric intake appear to play a part in the response. When rats were fed on a low thiamine ration (0.5 mg./kg. of diet), growth at the end of four weeks was 55 Gm. When penicillin was added, a weight of 115 Gm. was achieved on the same ration in the same period.[15] With chlortetracycline, the weight was 76 Gm.; with streptomycin 69 Gm.; with chloramphenicol and oxytetracycline, there was no response.

Choline deficiency results in a reduced feed intake and loss in weight. Also observed are acute involution of the thymus, kidney damage, and disturbance of the adrenal cortex, the latter probably secondary to the kidney damage.[2] Secretion of ACTH was altered by choline deficiency, being increased in young rats but decreased in adults.

Pyridoxine is essential for optimal adjustment to cold and other stressor stimuli. A deficiency is followed by reduced feed consumption, and the lower caloric intake immediately becomes an added factor. Deficiency (in rats and mice) is followed by marked atrophy of the thymus and by impaired formation or utilization of the cortical hormones, and probably of proteins generally.[2] This impairment is particularly marked when greater than normal amounts are required, as under conditions of stress.

Vitamin K deficiency may appear as an effect of stress that interferes with its absorption or utilization. Excessive vomiting is mentioned as a factor in human nutrition.[6] Vitamin K is required for the formation of prothrombin and other plasma proteins but does not become a part of the molecule of any.

Ascorbic acid is involved in the production and secretion of the adrenal cortical hormones.[5] A wide variety of stressor stimuli may increase the demand for vitamin C and thus deplete the supply. Deficiency of ascorbic acid results in adrenal hypertrophy and depletion and interferes with the formation of adrenal hormones of the cortisone type. Supplemental feeding of ascorbic acid (25 to 50 mg./day/rat), in deficiency, significantly increases resistance to cold, improves the utilization of cortical hormones, and can prevent the typical enlargement of the adrenals otherwise seen as response to long exposure to cold. It helps spare the hormones to such a degree that the normal production will meet the added demands resulting from stress. In so doing, it accomplishes the same result as administering ACTH.

Vitamin A is related to pituitary-adrenal function, but the precise nature of the relationship is not clear. Fat is needed for the absorption of carotene and vitamin A.[6] Carotene is found in the adrenal glands of the horse, dog, hog, and guinea pig. The adrenals of man and the rat contain large amounts of vitamin A but no carotene. The amount present is decreased by stressor stimuli. A deficiency may interfere with the formation or secretion of corticosteroids and thus become a factor in relative cortical hormone deficiency.

AMINO ACIDS

Although the influence of protein on the pituitary-adrenal system under resting conditions has been controversial, and some data on this point continue to be conflicting, the question appears to be answering itself as regards conditions of stress. The low protein rations impair the ability to synthesize gonadotrophins[4] and possibly other proteins, and this is accentuated under conditions of stress, and especially when certain of the vitamins are present in marginal or submarginal amounts, as previously mentioned.

Low protein rations prevent hypertension in the partially nephrectomized rat,[22] a condition that invariably occurs when ACTH is administered or when a high protein ration is fed. Since the response is approximately the same for both treatments, it is postulated that low protein rations interfere with the adrenal activity by depressing synthesis of the hormones or by interfering with their discharge from the gland. When the same amount of ACTH is administered to the rat on a high protein ration, no response is noted, indicating that a normal amount of adrenal hormone is already available. These authors also concluded that no single or specific amino acid was responsible, and it has been suggested[4] that the integrity of the hypophysis may be a determining factor.

In another test, it was concluded that the adrenal response to acute, short-lasting stress is also conditioned by the amount of protein or carbohydrate in the diet.[23] This was based on the observation that adrenal enlargement, cortical lipoid discharge, and lymphatic carioclasia were maximal in rats fed high amino acid diets and then exposed to stressor stimuli—cold, muscular exercise, formalin, and methane. When colchicine was the stressor agent, the response was greater in the rats on high carbohydrate rations.

Methionine is one amino acid that has been studied and found to be an important factor in the metabolism and utilization of nitrogen.[16] For example, nitrogen retention was increased by 7.5 per cent when a ration adequate in all nutrients except methionine was brought up to normal level. The response was even greater when antibiotics were also added, in fact, the effect of the combination closely approximated the sum of their individual effects. In other words, there was no evidence of a sparing effect of one substance for the other.

UNIDENTIFIED FACTORS

Stress increases the need for the substances commonly grouped under this heading,[4] and usually considered to be three or four in number—whey, fish, grass juice, and inorganic. The latter may be a part of any or each of the other three. The precise role of these factors is not clear, but they do delay the stage of ex-

haustion by prolonging the stage of resistance—probably by neutralizing toxins that are produced in more than normal amount during the period of greater activity resulting from adrenal-pituitary stimulation.

These new growth factors are supplied in varying amounts by a wide range of natural feeds, and supplemental amounts may be added to the ration in other substances for maximum efficiency and during periods of stress. Whole liver is an excellent source for human use, but too expensive for animal rations. The fermentation solubles, fish meal and fish solubles, whey, and alfalfa meal are widely used for the respective types, with yeast and antibiotic mycelium also finding a place.

MISCELLANEOUS FACTORS

Minerals may play an important part in minimizing stress.[4] A deficiency of sodium and of potassium causes pituitary-adrenal changes similar to those observed in stress, and a decreased production of desoxycorticosteroid-like hormone. Excessive potassium produces about the same result, however, so a matter of balance or equilibrium appears to be involved.

Adrenal hypertrophy and other manifestations of the general adaptation syndrome have also been caused in the rat by such diverse factors as chronic overfeeding, thirsting, and excessive consumption of cabbage and/or water.

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Possible Cancer Hazard Presented by Feeding Diethylstilbestrol to Cattle

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Introduction of the powerful drug diethystilbestrol into the nation's food supply prompts consideration of the actions of this compound. It is known to induce cancer.[1, 2] It has also been found to stimulate weight gain. For the latter reason, there has developed a practice of administering this drug to poultry and beef cattle in the United States.[3]

It has been estimated that more than 30 million chickens per year are implanted with pellets of diethylstilbestrol and that approximately half of the feed-lot cattle in the country are now given feeds to which this drug has been added. In poultry, the pellets are intended to be inserted at the base of the skull on the supposition that this part will be discarded and not eaten. But among nine lots of poultry coming into the New York market and examined by inspectors of the Food and Drug Administration, about 35 per cent of the birds were stated to contain such pellets in the neck. Similar findings emerged from subsequent investigations, and the residual diethylstilbestrol was reported as 3 to 24 mg. per bird.[4] It has been claimed that no appreciable quantities of diethystilbestrol can be demonstrated in the tissues of cattle fed this drug,[3] but this claim may convey a false sense of security for reasons subsequently given in this presentation.

Diethylstilbestrol can be made under conditions involving exposure to a temperature of 428 F. for several hours.[5] The temperature recommended for roasting chickens is 350 F. Temperatures of only 140 to 180 F. are found in the interior of roasts of beef.[6] From these figures, it is apparent that diethystilbestrol would not be destroyed by cooking and could be conveyed to consumers of meat containing it.

It thus seems pertinent to review the effects that this compound can exert. Administration of estrogens, among which diethylstilbestrol is one of the most potent, has led to a wide range of pathologic changes in human beings and in animals. In mice, rats, or guinea pigs, estrogens can induce polyps, fibroids, and cancers of the uterus, cancers of the cervix, cancers of the breast, hyperplasia of prostatic stroma and of endometrium, tumors of the testicle and hypophysis, and leukemias.[2]

In the experience of one of us (R.I.), uterine tumors have frequently been found in guinea pigs given as little as 1.5 mg. of diethylstilbestrol or other estro-

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gens in subcutaneously implanted pellets.[7,8] Indeed, tumors have resulted in guinea pigs exposed to as little as 8 μg. (0.008 mg.) of diethylstilbestrol per day.[7] Lower dosage levels have not yet been tested in guinea pigs, but in current experiments a pellet removed from a guinea pig one year after implantation has been found to retain sufficient activity to induce a tumor upon reimplantation into another animal.

Investigators at the National Cancer Institute[9] have reported that cancers of the breast can be induced in mice by as little as 0.07 μg. of diethylstilbestrol per day. This dosage sufficed to induce breast cancer in about 50 per cent of male mice, who do not develop such tumors in absence of exposure to an estrogen. A total dose of 30 μg. was effective in these experiments in which the drug was administered in a subcutaneously implanted pellet. These and other investigators found that diethylstilbestrol also induced tumors readily when given to mice by mouth. Normal mice excrete between 0.05 and 0.1 μg. of estrogen per day.[10] So delicate is the physiologic balance that exposure to an approximately equal additional amount (0.07 μg.) of estrogen (diethylstilbestrol) per day suffices to induce cancer, as previously mentioned.

From these findings, it is obvious that the cancer-producing dose of this drug approaches the infinitesimal. Claims that no appreciable quantities of it can be demonstrated in tissues of cattle to which it has been fed must therefore be carefully scrutinized as to the sensitivity and accuracy of the test methods. An equally cogent consideration is that the fundamental mechanism of cancer induction by diethylstilbestrol is not understood. Many tumors are known to be caused by viruses.[2] In mice, cancer of the breast has been traced to a virus, but, for its cancer-producing activity, this virus is dependent upon stimulation by estrogens, such as diethylstilbestrol. This virus remains in the tissues and exerts its neoplastic effects long after cessation of administration of estrogens. Absence of detectable estrogen in the tissues of animals treated with or fed such a substance thus offers no assurance of the absence of a cancer hazard in such tissues.

A great body of evidence shows that cancer-inciting chemicals can exert their effects in catalytic quantities, inducing changes in cells which are mediated by unknown substances transmitted from cell to cell long after the original cancer-inciting material ceases to be demonstrable in the tissues.[2] No assurance of the absence of such substances can be offered consumers of tissues from animals treated with or fed a carcinogen, such as diethylstilbestrol.

In human beings a variety of pathologic changes have been found to follow administration of estrogens. In women well past the menopause, the course of breast cancer is slowed by estrogens, but in somewhat younger women it is accelerated.[11] There are claims for primary initiation of cancer in women by estrogens,[12] but these have been few in contrast to the great number and diversity of tumors following estrogenic treatment of animals. Now it has been found from animal experiments that the spacing of doses greatly influences the yield of tumors. Most important, it has been learned from animal work that intermittent administration of very large doses of estrogens is far less effective in inducing tumors than is a continuing exposure to an extremely minute dose. This phenomenon has been repeatedly observed by one of us (R.I.) in experiments conducted over a period of nearly 20 years with Lipschutz.[7] It is a continuing exposure to extremely minute doses that is to be feared from the introduction of estrogens into the food supply.

A prime consideration is the long period of time that elapses between first exposure to a carcinogen, such as diethylstilbestrol, and eventual appearance of a tumor. In animal experiments, exposure is customarily begun early in life and the majority of tumors arise when the animals are old. Experience in the results of administration of estrogens to human beings has been largely limited to treatment of conditions arising fairly late in life. By comparison, the majority of human beings thus far exposed would complete their life span before passage of sufficient time to observe a carcinogenic effect of estrogens. The introduction of estrogens into the food supply, however, presents the problem of exposure of human beings from birth onward. That human beings are not immune to the cancer-inciting action of estrogens is shown by the fact that there are on record some 17 cases of cancer of the breast in men given estrogens, including diethystilbestrol.[13-15]

Figures were presented to this symposium[16] which, according to our understanding, represent the finding of about 1 μg. of diethylstilbestrol per pound in the tissue of a steer given the prescribed amount of 10 mg. of this drug in feed. One pound of such tissue would thus contain about 14 times the amount of diethylstilbestrol needed as a daily dose to produce cancer in mice, for, as previously stated, 0.07 μg. of diethylstilbestrol per day sufficed to elicit cancer in about 50 per cent of mice. Furthermore, claims for the absence of diethylstilbestrol in tissues have been based on a method whose limit of sensitivity[5] is, according to our calculations, in the range of 1 μg. per 1.1 lb. This means that a pound of meat, certified as free of diethylstilbestrol, could contain nearly 14 times the amount of this drug necessary to induce cancer by a daily dose to mice. In the case of market poultry found to contain up to 24 mg. of diethylstilbestrol per bird,[4] one is dealing with an amount roughly equivalent to 342,000 times the daily dose necessary to produce cancer in mice.

It would therefore seem prudent that further careful consideration be given the matter of adding to the cycle of food supply a drug known to initiate or aggravate a serious disease. A panel discussion of this presentation follows the Bibliography.

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PANEL DISCUSSION: Feeding Diethylstilbestrol to Cattle

Moderator (Dr. Henry Welch): Thank you, Dr. Smith, for a most interesting presentation. I am sure that we will have some rebuttal on several of the questions raised by Dr. Smith. Several questions occurred to me as I listened to the presentation, and I am sure others need answering as well. In our audience I know that there are several people who are well known for their studies in this field. In order to open the discussion, I think perhaps I will call on a few of them to give their views concerning Dr. Smith's presentation. Is Dr. Hines here?

Dr. Hines: Before commenting on the paper, I think perhaps I should give you a little of the background for my speaking.

I was a practicing physician for a number of years before I joined the medical research group at Eli Lilly & Company. I have been interested in stilbestrol since it first came under clinical investigation in about 1938.

By 1940, there was a tremendous amount of interest in this compound, and in order to get all of the material together and have a unified presentation of it for the Food and Drug Administration, the Administration requested that a committee from the pharmaceutic industry be formed to present the clinical results in a unified pool. I happened to be appointed as chairman of that committee, and eventually we submitted to the Food and Drug Administration clinical reports covering 8000 women who had received diethylstilbestrol. Eighteen hundred of these women had received it continuously for a minimum period of six months, and some of them had received it for as long as two years. That was in 1941. We have now had almost 15 years' additional experience with exposure of human beings to this substance and an even longer period of experience with exposure to the natural estrogens given therapeutically. The natural estrogens came into use in the early 1930's, so we have between 20 and 25 years of experience with them.

The thesis that was presented here this afternoon is not a new one. A good part of it was first presented when estrogens came into use almost 25 years ago. This picture was well formulated by the time diethylstilbestrol came along in 1940. The fact seems to be that in the last 15 years, there has been no evidence to sub-

stantiate this thesis. In 25 years, there has been no increase in the incidence of cancer that could be attributed to the use of estrogens.

Now, I may say that it is important in considering this subject and that we have some definite ideas of the quantitative aspect of it. It is well known, I think, to a great many in the audience that particularly in the endocrine field you cannot carry over from one species to another the experimental results that are obtained. This is particularly true in carrying over to a human being the effect observed in an animal. In many ways this has been disappointing, because some of the results obtained from the animals led to therapeutic hopes that have not been realized.

Certainly the results that have been obtained in cancer in mice are very interesting. However, mice are not men. There are two kinds of differences. Men, on the average, weigh about 3500 times what a mouse weighs. In addition to that, there are qualitative differences, too, so that the analogy, while interesting, is perhaps not too relevant.

It should be pointed out that there are estrogens in a great many foods. They are present in microgram quantities. Women produce estrogens in very significant amounts, milligram quantities; men produce them, too. Men do not produce as much as women, but all normal men constantly have estrogens circulating in their bodies. According to my interpretation of the tests on treated beef, 1 μg. of stilbestrol per pound of beef will give a positive test, not a negative test. Since the tests are negative, we can assume that people who eat a pound of treated beef a day will be exposed to less than 1 μg. of stilbestrol per day more than if they ate untreated beef. That amount is very small; it is microscopic in comparison to the amounts of estrogen that are normally circulating. So if one assumes therefore that one is taking in this quantity of estrogens, one is not making any appreciable, or, as a matter of fact, hardly measurable, increase in the supply of estrogen that is already present.

MODERATOR: Thank you, Dr. Hines.

I would like to call on Dr. Herbert Luther of Chas. Pfizer & Co., Director of the Agriculture Research Farm, who has had considerable experience in this field. Dr. Luther.

DR. HERBERT LUTHER: I would like to quantitate some of the comments that Dr. Hines made regarding natural estrogens content of foods and would like to ask Dr. Smith if he had any comment particularly with reference to the intake of estrogen in this regard.

It is well known from published information that there is a significant but variable estrogen content of alfalfa, which is consumed in large quantities by ruminating animals. It is also known in Australia that the subterranean clover is sufficiently high to give some visable estrogenic side effects; Dr. Andrews and his colleagues of Purdue are going to publish soon some work on the estrogen content of 50 varieties of alfalfa that they have studied in the midwest area of the United States. They will report that their estrogen content varied from 0 to 27 μg./lb.

In addition, the estrogen content of the natural tissue of the animal, I think, perhaps should be considered in our work. We have found, for example, that the fat content of the normal steers who have had no access to diethylstilbestrol continually runs more than 4 parts per billion.

Similarly, with liver, average values for natural estrogenic activity of several control steers that have received no stilbestrol run higher than 1 part per billion.

Control kidney has been more than 1 part per billion and the lean meat from 0.7 to 1.2 parts per billion.

With reference to the statements that we are exposed to natural estrogens in our food, I think this is quite true, as Dr. Hines suggested. This is true, for example, in mother's milk. We have been studying the estrogen content of normal milk produced in dairies where naturally the estrogen content will vary depending on the herd and also the stage of pregnancy, but we were finding that the average content of dried milk is running on the order of 5 to 8 parts per billion, which is considerably higher than the figures we are discussing here, and Dr. Turner of Missouri, in unpublished data, has obtained a doubling of the uterine weight of mice that have been fed normal fluid whole milk picked up on the open market.

MODERATOR: Thank you, Dr. Luther.

Now I would like to call on Dr. Franz Gassner, Director of Endocrinology Research at Colorado A. & M.

DR. GASSNER: As head of the Endocrine Section at the Colorado A. & M. Experiment Station, Fort Collins, Col., I am in charge of research projects that deal with the functional relationships of hormones and other agents to reproduction and nutrition of livestock. Following the disclosures by the Iowa State Experiment Station of the startling results obtained with the addition of small amounts of diethylstilbestrol to cattle feed on feed-lot performance of steers, it became necessary to determine if and to what extent this method of feed medication would be applicable in our western beef-producing areas. Of particular interest was to find out whether this procedure as recommended for the midwest could also be applied to fattening procedures practiced in the west and far west because of differences in feedstuffs used.

We have just completed large feeding trials involving more than 2000 animals. Every effort was made in careful and critical determination of the efficacy and the economy of the use of diethylstilbestrol in the fattening of cattle and particularly of the safety of use of such treatment to the animal, and to the human consumer of meat products derived therefrom.

To determine the presence of any hormone residue in edible parts of the beef carcass, we have employed an extremely sensitive assay method that utilizes the uterine weight response of the immature mouse and that has been approved by the Food and Drug Administration. On the basis of large series of assays using more than 3000 mice, we were able to confirm fully the sensitivity and validity of this assay method as originally reported and as subsequently modified. In no case were we able to detect any estrogenic response in the mice that could be ascribed to diethylstilbestrol administered in cattle feed. A special effort was made to determine any possible side effects on cattle as every animal was followed through slaughter. We obtained more than 600 sets of accessory sex organs, such as seminal vesicles and prostates. On the basis of changes in weight and histologic appearance of these organs in comparison with those of the untreated controls, in no instance were we able to detect any significant signs of estrogenic influence. Measurements of teat length and mammary development were likewise negative. These results by and large confirm the findings of numerous experiment stations, including the United States Department of Agriculture Research Branch at Beltsville, Md.

We are also fully in accord with the information given by Dr. Luther. We have frequently noted that muscle, liver, and, occasionally, kidney and abdominal fat

of cattle that had not been fed diethylstilbestrol elicit a measurable estrogenic response equivalent to that obtained with 2 to 8 μg. of diethylstilbestrol when tested on the immature mouse uterus. Since these animals did not receive any medicated feed, it is evident that there exists a rather wide distribution of estrogenic materials in natural feeds, as has been pointed out by previous speakers.

It has been adequately demonstrated that an appreciable amount of estrogens or their precursors are contained in green, leafy vegetables and forage, such as lettuce, subterranean clover, and alfalfa, and in grains, such as soybeans, corn, and their by-products. It is a common observation that when open heifers are allowed to graze on cereal grass pastures which are in the young, succulent stage of growth, estrus or sexual heat is often induced rather promptly. All of this points to the fact that our livestock is continuously exposed to the intake of sometimes rather high levels of estrogenic materials in feedstuffs. The human is no exception, including the baby being fed Pablum, for example, which contains alfalfa. Thus it seems that man and animal alike are forced normally to maintain a high threshold of response to estrogens and that the human is rendered relatively insensitive to the infinitesimally small amounts of hormone that possibly could be ingested with meat products. Finally, it has been adequately demonstrated that with the highly sensitive control methods employed by the Food and Drug Administration, the Public Health Service, in cooperation with a host of qualified institutional investigators, are fully capable of maintaining adequate safety measures for the use of medicated feeds. Therefore, the concern expressed by the authors of the paper under discussion becomes quite pointless and unwarranted.

MODERATOR: Thank you, Dr. Gassner.

I would now like to call on Dr. T. C. Byerly of the United States Department of Agriculture, who has had experience in this field.

DR. BYERLY: I should like to direct my remarks to one particular kind of neoplasm, one of the few known to be caused by a virus—the avian leukosis complex.

In 1942 I speculated that the continuous exposure of the laying hen to estrogens might explain the fact that nonbroody hens died more frequently within the avian leukosis complex than broody hens.

It is an established fact that hens have a much higher incidence of the avian leukosis complex than do cockerels. However, capons have a still higher incidence. Within a few years time after I made my speculation, Dr. Burmester, working in the Regional Poultry Laboratory in East Lansing, with Dr. Nelson published the effects of experimental trials on castration and sex hormones on the incidence of lymphomatosis and, if I may, I should like to read from that summary:

". . . White leghorn chickens of both sexes were used to determine the effect of castration and implantation of diethylstilbestrol and testosterone proprionate upon the incidence of lymphomatosis.

"Castrated males, whether innoculated with the blood of donors having lesions of lymphomatosis or non-innoculated had a significantly higher incidence of lymphomatosis than normal males of the same breeding.

"The castrated female lot also had a higher percentage of lymphomatosis than the normal controls. These differences among the females, however, were not significant. Capons treated with female hormones had a significantly *lower* incidence

than untreated capons, although no significant effect was demonstrated on normal males. Males and capons treated with the male hormone had a significantly lower incidence of lymphomatosis than untreated males and capons. The results obtained suggest that the male hormone increases the resistance of males and capons to lymphomatosis and this in part probably accounts for the fact that the incidence of this disease is usually much lower among males than among females.

"Speculations, it seems to me, are a necessary part of scientific research. I think a research man has a responsibility to test his speculations. Mine on estrogens and the avian leukosis complex (lymphomatosis) were tested and found wanting. It has been ten years since we considered it worth while to study the relationship of stilbestrol to the avian leukosis complex."

MODERATOR: I would now like to call on Dr. Delbert Bergenstal who is with the Endocrinology Branch of the National Cancer Institute.

DR. DELBERT BERGENSTAL: I have been interested in many of the comments that have been made regarding Dr. Smith's paper, and I would simply like to comment mainly along the lines on which Dr. Hines has spoken.

There can be little doubt that sex hormones, particularly the estrogens, have a profound effect upon the growth of normal tissue. They can increase the rate of growth in normal tissue manyfold but whether this growth can be neoplastic has yet to be proved.

There is a rich and large background of animal experimentation, which has been referred to by Dr. Smith and other speakers, and I think we can look at this critically for just a moment and then go to the human data that we have available.

This afternoon, Dr. Smith mentioned that certain strains of mice given a minute amount of estrogen will develop breast cancer. This is indeed true, but there are many other strains of mice, rats, guinea pigs, monkeys, rabbits, in which no carcinoma of the breast develops during the administration of estrogens. An example of this is an experiment done with guinea pigs in which minute amounts of diethylstilbestrol produce fibroid tumors of the uterus. A similar experiment was done with monkeys where similar doses of estrogen were given. This estrogen was administered for a period up to 10 years to some of the monkeys, and no tumors were found in the breast or the genitourinary tract of these monkeys.

We are thus left with a large volume of clinical data in humans to which we can look for evidence as to whether hormones, particularly estrogen hormones, can produce neoplasia. I only wish that these data were as carefully compiled and studied as the animal experiments, but, as is frequently the case in clinical research, it is collected from many sources and difficult to evaluate fully. Yet, I do think we have enough that we can draw certain conclusions.

First of all, I would like to say that in my own mind I do not believe that there has been proved as yet a single case of a human neoplasia being produced by estrogen administration.

Dr. Smith has made reference to the presence of breast cancer in patients with carcinoma of the prostate who have been treated with estrogens. The number of cases in the literature are very few and there has been at least one case observed in which there was observed a carcinoma of the breast and a carcinoma of the prostate occurring in an individual who had not received estrogen therapy. It may well be that a more careful survey of the literature would produce other cases of

this type. It also may well be that this relationship between carcinoma of the prostate and carcinoma of the breast is very similar to the relationship we have seen between carcinoma of the breast in the female and carcinoma of the cervix and uterus. It is interesting here, however, that if Dr. Smith feels that this carcinoma was produced by the estrogen, that it is an example where large amounts of estrogens were given for a relatively short time, which is quite in contradistinction to what he mentioned earlier, that minute amounts of estrogen are necessary for a long period of time before neoplasia develops.

Other critical data that we may turn to would be the very excellent report of the Therapeutic Trials Committee of the American Medical Association. Here, an evaluation was made of carcinoma of the female breast and the genitourinary tract in women treated with estrogens. These patients were treated for varying periods of time, for as short as three months or as long as three years. The doses used were massive in terms of what we are talking about here today. In no case was a new tumor found to occur or in no case was the incidence of the appearance of another lesion in the contralateral breast greater than that found in the control series.

As Dr. Hines has pointed out, we now have about a 20 year period in which estrogens have been used for many and varied gynecologic disorders and menstrual disorders, and I believe no one as yet has been able to prove the existence of a tumor directly related to the administration of these estrogens. Throughout the entire reproductive period of a woman, there is a continuing change in the concentration of estrogens throughout the cycle, reaching levels far in excess of anything that would occur in the meat products that we have been talking about and dropping to levels that are within this range or even less.

I would be the first to admit that a great deal more research has to be done, not only with estrogens but with all types of hormones in attempting to find out more about how these various potent materials regulate growth processes, not only cancer growth processes but normal growth processes as well. Until we have more evidence, then I think we must all keep an open mind as to the potential effects of these hormonal substances on growth.

MODERATOR: Thank you, Dr. Bergenstal.

I am sure that there are a lot of people in the audience that would like to comment. I am going to call on Dr. Holland, who is our medical director in this organization, because I think we in the Food and Drug Administration are being criticized somewhat in this area because of the release of stilbestrol. I can assure you that there was a tremendous amount of reluctance on our part in releasing it and a great deal of detailed experimental work was required before it was released. I would like to tell you what we have been called for being so reluctant, but I believe you can imagine.

Before I call on Dr. Holland, whom I would like to ask to summarize the position of the Food and Drug Administration, are there others in the audience who would like to discuss either the paper of Dr. Smith or those of the people who have discussed his paper? If not, Dr. Holland, will you please state the position of the Food and Drug Administration.

DR. HOLLAND: I think we have had an excellent example of the kind of complex and extremely important problems that a technical agency, a scientific agency, like the Food and Drug Administration must face frequently.

Our position, I think, is very clear. All of the data, all of the work, all of the literature we have had occasion to study and review over a period of many, many months preponderantly led to the conclusion that diethylstilbestrol as proposed for use in cattle feed was perfectly safe.

It is the general purpose of the Food, Drug, and Cosmetic Act to protect the public health. We believe that the preponderance of the evidence, and we must deal in fact rather than speculation, indicates that the decision was a proper one.

We, of course, do not have a closed mind, nor shall we ever have. We must deal impartially with scientific evidence as presented, and if there is information available that is pertinent to this question, whether it be now or at any subsequent date, we will always be willing to consider it. But it, like all other evidence considered, must be subject to the critical examination of scientists the world over.

MODERATOR: We still have a minute or two more for this paper. Dr. Smith, would you like to make any further remarks after having heard the discussion of your paper?

DR. SMITH: I believe the major point that has been brought up is the old problem that we are always faced with in evaluating any toxicologic problem, and that is how much is bad and who is it bad for. Material that is bad for mice may not be harmful to guinea pigs. A material that may be harmful to guinea pigs may have no effect on rabbits. It is difficult to carry over, as the first commenter said, experience from one animal species to another.

However, it would appear that in the case of the action of stilbestrol in inducing tumors, we are not required to work completely in the dark in taking experience from animals and attempting to evaluate possible significance for man. Stilbestrol produces tumors in a number of species of animals, and it produces a great number of different types of tumors. And, in man, I understand that the majority of authorities do accept the breast cancers that have occurred in men treated with estrogens, among which stilbestrol has been one, as tumors initiated by the estrogenic drug.

Now, in the matter of dosage, we are concerned here not with a substance, such as a common poison, all of which have threshold values. You take a little, and, if it does not do any great harm, you excrete it, get rid of it, and no harm has been done. Threshold values can be set up for almost any material, and, of course, most materials do have some toxic action if too much is taken, but in the case of the carcinogens we are concerned with substances that can have irreversible effects. The effect of the small dose is not forgotten by the animal. It is a peculiar, special pharmacological situation that the carcinogens present. I would like to read a sentence or two from resolutions adopted by a symposium on cancer prevention held by the International Union Against Cancer in 1954. There were representatives from many countries there and these resolutions were adopted unanimously. They have been published in the Acta Union internationale contre le Cancer, Volume XI, No. 1, pp. 72-76 and they read:

"Evidence exists to show that the time of appearance of tumors after exposure to carcinogenic agents is, within limits, dependent upon the dose and the frequency of exposure, but small doses and often a single dose of carcinogenic agents may elicit tumors, notably after prolonged latent periods. In view of the latter finding, and in view of the summative carcinogenic effect of repeated small doses, concepts

of safe threshold doses are dubious where complete control of a hazard involving exposure to carcinogenic agents is desired."

Now, we simply do not know the dosage of estrogens that may be effective in producing tumors in man. All the experience that we have and all of the experience that has been cited here this afternoon depends on experience gained over a period of 15 years in individuals who, in a great majority of cases, received these drugs, either in middle or late life. We cannot forget that time is a most important element in the action of any carcinogenic agent. A carcinogenic material administered toward the end of an animal's life may not have time to manifest its carcinogenic activity. There is a great deal of evidence to demonstrate that point. We do not know what the tumor-producing dose may be, if we are dealing with the administration of this particular drug to individuals from birth onward.

MODERATOR: Thank you, Dr. Smith. This has been very interesting, especially to me. I am quite convinced after hearing most of it that as of now I must stop eating. Apparently the amount of stilbestrol that you get in dairy milk is higher than the amount allowed in beef; its presence in cereal and the amount a steer gets in feed is less than some normally carry, and that cuts out all of the beef. It leaves little food that contains no stilbestrol. The critical points of the paper and discussion seem clear. Diethylstilbestrol can induce tumors in some mice but not all strains, in some other animal species, but not all. This drug is present in many of the foods we eat daily and it is present in mother's milk. The quantities present in these foods far exceeds the amount allowed to be present in beef fed diethylstilbestrol-medicated feed. These are the facts, the rest appears to be theoretical considerations.

I think we will have to close the discussion and go on to the next paper on the program.

Responsibility of Manufacturers for Legal Labeling of Drugs in Animal Feeds

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In view of the statutory definitions contained in the Federal Food, Drug, and Cosmetic Act (in its present form), so-called "medicated animal feeds" must be regarded as drugs, and primarily labeled as such in compliance with legal requirements for the proper labeling of drugs. To label such articles as animal feeds containing one or more drug ingredients is to invite the penalties of the law for adulteration of food. These may seem to be rather strong statements. However, let us examine the definitions as the United States Congress phrased them in 1938.

"The term 'food' means . . . articles *used* for food or drink for man or other animals. . . ." Notice that I have italicized the word "used." Certainly nux vomica, poke root, arsenic, fenugreek, sulfonamides, nitrofurans, coccidiostats, synthetic estrogens, antibiotics, and other prophylactic, therapeutic, and growth-stimulating substances are not used for food or drink for man or other animals. Neither can they be considered as normal components of a "food."

"The term 'drug' means . . . articles *intended* for use in the diagnosis, cure, mitigation, treatment, or prevention of diseases in man or other animals; and . . . articles (other than food) *intended* to affect the structure or any function of the body of man or other animals. . . ." Notice that I have here italicized the word "intended," which does not appear in the previous definition. Thus a food may be legally actionable as a drug because of false or misleading claims made for it by its manufacturer, but the reverse is not tenable under statutory definition.

The law provides that "A food shall be deemed to be adulterated . . . if it bears or contains any added poisonous or added deleterious substance . . . [which is not] required in the production thereof or cannot be avoided by good manufacturing practice. . . ." Since all the drug ingredients mentioned previously must be classified as "toxic or deleterious substances," it is apparent that a so-called "medicated animal feed" labeled primarily as feed containing a drug must be regarded legally as an "adulterated food" under present Federal law.

In the light of the foregoing interpretations of the law, the Food and Drug Administration has consistently advised manufacturers of so-called "medicated feeds" to designate such articles by trade or brand names that are not misleading and that inform the purchaser that the products are drugs primarily intended for disease prophylaxis or therapy or intended for some effect (other than that of a food) on the structure or function of the animal body. Examples of suitable desig-

nations under the Federal law are: "Blank's Sulfaquinoxaline Mixture, for controlling outbreaks of poultry coccidiosis," "Blank's Arsanilic Acid Mix, for stimulating rate of growth in chicks and poults," "Blank's 0.0011% Diethylstilbestrol Mix, for accelerating weight gains in beef cattle," and "Blank's Phonothiazine and Nicotine Mixture, for removal of large roundworms (ascarids) and cecal worms from chickens."

In compliance with the requirements of the Act for proper labeling of drugs, the label of a "medicated feed" should declare the active drug ingredients by themselves in a prominent and conspicuous manner. The declaration of the active drug ingredients by their common or usual names may be followed by a revelation that they are "Incorporated in (or mixed with) Blank's Steer Pellets," "Blank's Broiler Starter," or "Blank's Hog Feed," as the case may be. In short, the feed formula becomes the vehicle or carrier of the drug ingredients in such preparations.

The Act provides that failure to reveal material facts in the labeling of a drug may result in its being misbranded by misleading labeling. To avoid contravention of this provision of the Federal statute and to satisfy the requirements of State feed control regulations, the labels of so-called "medicated animal feeds" must declare the guaranteed analysis of the feed base and list the feed ingredients in the order of their decreasing importance. Unless the principal display panel (usually the front or obverse side) conspicuously presents the directions and warnings for proper use of the article, the label should bear a statement such as "IMPORTANT: Carefully follow directions (and warnings) for use on the reverse side."

In addition to declaring the active drug ingredients properly and providing adequate directions and warnings, other mandatory information required by law on the label of a drug includes an accurate statement of the quantity of the contents and the name and place of business of the manufacturer, packer, or distributor.

Since the law deems a food or a drug to be misbranded if its labeling is false or misleading in any particular, manufacturers of so-called "medicated animal feeds," as well as manufacturers of the drugs or drug premixes to be mixed with animal and poultry feeds, should be particularly careful not to make claims for the active drug ingredients or the feed formula vehicle that are not well founded in fact or that cannot be supported by sound scientific evidence. Even in this era of advanced knowledge and progress in medical and nutritional science, many manufacturers attempt to justify questionable statements and claims by producing large numbers of "unsolicited testimonials" from satisfied users of their products. Naturally, letters that are uncomplimentary about the products are seldom produced. Testimonials of enthusiastic customers mean little or nothing from a scientific standpoint. Many products that have been proved worthless when subjected to critical tests are very often highly recommended by farmers and stockmen who are not trained in judging the correlation between causes and effects in connection with the prevention and treatment of diseases.

Indirect and ambiguous statements that *may* mislead should be avoided, as also should designs and devices that may create an unwarranted impression. Interpreting the general misbranding provisions of the law the United States Supreme Court said: "The statute is plain and direct. Its comprehensive terms condemn every statement, design, and device which may mislead or deceive. Deception may result

from the use of statements not technically false or which may be literally true. The aim of the statute is to prevent that resulting from indirection and ambiguity, as well as from statements which are false. It is not difficult to choose statements, designs, and devices which will not deceive. Those which are ambiguous and liable to mislead should be read favorably to the accomplishment of the purpose of the act."

Very much in the same vein a United States District Court, at the conclusion of a trial involving an allegedly misbranded drug, charged the jury as follows: "At the outset, gentlemen, the question is submitted to you, and it is for your consideration, as has been correctly said, largely in the light of good sense, as to what this label means; and you are to test that out by taking the language of it and imparting to that language the meaning of the words, singly and together, that would be conveyed to you as ordinary men; not as men who are skilled in medical, chemical, or pharmaceutical science, capable of making nice distinctions or nice discriminations, but rather the meaning that comes to you as ordinary men unskilled but seeking, we will assume, some sort of remedy or remedial help for the afflictions that flesh is heir to. Now, in that connection you should examine this language in the light of the purpose of this law, which is to protect humankind against the consequences of human weakness, or human frailty, or human credulity or the disposition to believe, or of human gullibility. You should examine it in the light of the disposition of the ordinary humankind to wish to believe in the potency of remedial agents to relieve them from ills from which they are actually or conceivably suffering."

These judicial pronouncements serve to emphasize the heavy responsibility to the public that must be assumed exclusively by the manufacturer who produces a preparation represented as beneficial to the health of groups or individuals, be they animal, fowl, or man. Remember that health cannot be sold by the bottle, box, or bag.

Please bear in mind also that the Food and Drug Administration is not authorized by law to share the manufacturers' responsibilities. Many of you know from long experience that the Administration cannot approve or condemn preparations that are subject to the provisions of the Federal Food, Drug, and Cosmetic Act. With very few exceptions, it can neither approve nor disapprove the labeling of such products. Yet we are constantly requested to do so, and in correspondence and personal discussions many representatives of the industry refer to products and labeling that we have "approved." Nevertheless, if possible, we are always willing to express an informal opinion on the legality of labeling when requested to do so and when furnished the complete facts concerning the quantitative formula and dosage schedule.

Future of Medicated Feeds

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Predicting what may happen in the future is always a hazardous undertaking regardless of the specific field involved.

We feel that it is quite safe to say that medicated feeds are "here to stay." No doubt there will continue to be new discoveries and developments, and no one can predict just how these new discoveries or their application may change present-day practices and methods.

Past experiences are sometimes an excellent guide in helping to forecast the future. This being the case, perhaps it is well to review briefly past trends in the medicated feeds field. As we all know, medicated feeds are not particularly new. We can recall that a number of years ago certain substances were added to feeds, primarily for worming purposes. The use of chemicals and drugs as an aid in preventing or controlling disease, such as coccidiosis, are not new. It is true that there may have been some question as to the effectiveness of some of these earlier remedies.

We were associated with a small feed manufacturing concern until 1945. At that time, only 10 years ago, this concern had not used any ingredients in its feed for disease control purposes. Antibiotics and other various growth stimulants were still unknown, as far as use in feed was concerned. Various tonics, powders, and other remedies were on the market at that time—primarily to be added to the drinking water.

The big incentive for the use of medicated feeds came about with the development of satisfactory and effective coccidiostats. Since that time, there has been a veritable parade of drugs and chemicals that have come into use for specific purposes. There is no reason to believe that this trend will not continue in the future.

What are the reasons that lead us to believe the use of medicated feeds will continue in the future and perhaps even on a larger scale than is now prevalent? We feel it is safe to assume that the trend of mass production of poultry and livestock will be accelerated in the years ahead. Specialized large-scale operations are becoming more commonplace every day. As a service to feeders, feed manufacturers will continue to make medicaments available that can be administered at proper levels to livestock through feed. It is generally recognized that feeders are saving millions of dollars through prevention of disease and are also realizing increased rates of growth and less risks in their operation due to performance

of medicated feeds. Earlier, there were many skeptics who questioned the basic soundness of this approach, but the great majority have now been converted.

Medicated feeds have brought on many additional responsibilities and problems for the feed industry. It is possible that some of these problems may become more serious in the future. Those not associated directly with the industry probably do not realize the extent or seriousness of some of these problems. Much closer cooperation between all departments of a feed plant are needed today. This is especially true of production, research, and quality control departments.

Chemical ingredients create special inventory problems that must be given careful attention. Production has become much more exacting and complicated. The absolute necessity of proper mixing cannot be overemphasized. These substances are used in very small amounts and must be mixed very carefully so that every spoonful of the feed is uniform. Additional personnel with technical training is necessary to check constantly for quality, uniformity, and potency. More care is needed to make sure that the feeds are properly identified for storage and shipment. Problems of clearing, labeling, and registering have become more complicated. The need for more concise, specific, and detailed feeding instructions also merits special mention.

The use of medicated feeds has hastened the necessity for better-trained salesmen and servicemen. Manufacturers have been willing to meet these problems in order to be of more service to their customers. To do this has involved more than the simple cost of the added medicament. We seriously doubt if any manufacturer of medicated feed has fully passed on this additional cost that could easily be justified.

The feed industry fully recognizes its responsibility and will exercise caution and judgment to see that all proper precautions are taken in the manufacture and sale of feeds containing chemical additives. In this respect, we will welcome the continued help and cooperation of Federal and State agencies, drug suppliers, educational groups, veterinarians, and others. With this cooperation, we are sure that all of us can face the future with confidence.

Hormonizing Poultry with Dienestrol Diacetate

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The practice of hormonizing poultry with synthetic estrogens has been well established during the past decade. Initially, this chemical caponizing was accomplished by using diethylstilbestrol pellets that were inserted subcutaneously in the neck of the bird at the base of the skull about three to five weeks before the predetermined date of slaughter. Later, alternatives to the pellet were paste or suspension dosage forms of diethylstilbestrol, which were injected in the same area at the base of the skull.

It is only a little more than a year since oral hormonizing with dienestrol diacetate was introduced on a commercial scale. Since dienestrol diacetate is relatively insoluble in water but soluble in oil, the initial product was marketed as a soybean oil suspension to be dissolved in additional warm oil prior to its incorporation in the feed by the feed manufacturer.

DRY MIX DEVELOPMENT

In order to avoid these additional steps in hormonized feed preparation, work was conducted to make a dry mix concentrate that could be incorporated in the feed more readily and that would be just as effective as the previously developed and thoroughly tested oil solution. The selection of a suitable carrier for such a dry mix involves the consideration of various factors, which may be summarized as follows.

Concentration of Dienestrol Diacetate (DD) in Dry Mix. It is desirable to use the minimum weight of carrier per unit weight of DD consistent with satisfactory results on the poultry and ease of handling by the feed manufacturer. Such minimum weight ensures minimum cost of materials, packages, storage, transport, and maximum production output. With these facts in mind, the concentration selected was 63.5 Gm. dienestrol diacetate per lb. of dry mix (14 per cent DD by weight) to be used for making 1 ton of final feed. This has the added advantage that it lends itself readily to the desirable practice of making a 1:10 premix (drug dilution) per ton of feed, the added dilution material being a portion of one of the regular ingredients used in the final feed.

Grain Size. Generally, a coarser grain is preferable from the free-flowing and nondusting points of view; however, this property must be correlated with product uniformity.

Fat Content. Higher fat content is preferable for dust prevention; however, it must not be so high as to cause stickiness and lumping.

Flowing Quality. It is important that the product should not form lumps in storage or transit, even at low temperatures. Furthermore, it should flow freely for ease of dilution in the manufacture of the hormonized feed.

Dusting Quality. It is highly desirable from the point of view of the dry mix manufacturer, the feed manufacturer, and the grower-user, that the product should form as little dust as possible in the various operations.

Uniformity of Dry Mix and Final Feed. Both the dry mix and the final feed must be uniform initially and must remain uniform even after extensive shaking in transit and handling. With this in mind, it is important to have a carrier to which the active ingredient adheres firmly.

Various promising carriers were examined with these facts in mind, and the results of these examinations are summarized in table I. These data indicated that the best balance of the desired qualities was embodied in a special grade of corn distillers' dried grains, and, accordingly, this was the carrier selected for the dry mix.

PERFORMANCE IN BIOLOGIC SCREENING TESTS

It is naturally desirable to evaluate the performance of such a product in a relatively short time before carrying out large-scale field trials to confirm its efficacy

TABLE I

Characteristics of Carriers for Dienestrol Diacetate

Product	Milo flour	Milo cones	Corn distillers' dried grains	Soya products A	Soya products B	Soya products C	Soya products D
Screen Analysis							
On 20 mesh	0	0	0.7	similar	0	0	0
Thru 20 mesh on 40	0.3	0	19.4	to B	—	1.7	30.2
Thru 40 mesh on 60	5.0	64.3	47.5		—	11.8	56.0
Thru 60 mesh on 80	16.0	18.1	26.5		0.5	20.5	7.0
Thru 80 mesh on 100	32.6	9.5	3.4		4.2	14.4	2.4
Thru 100 mesh on 200	31.8	6.5	1.0		39.5	25.6	2.1
Thru 200 mesh	13.7	1.8	0.1		54.8	25.9	2.0
Fat content, %	2.7	2.8	7.0	5.5	0.6	0.6	0.6
Density, lb./cu. ft.*	31–40	30–	26–35	27–35	33–47	45–54	45–49
Flowing quality†	MF	FF	FF	SL	MF	FF	FF
Dusting quality‡	ND	ND	ND	ND	VD	FD	ND
Stratification	—	none	none §			none	none
Adherence to carrier	OK	OK	OK	Fair	Poor	Poor	Poor
Moisture content, %	12.7	12.2	6.0	9.0	6.0	11.0	11.0

* Lower values were poured in loose; higher values were shaken down.

† MF = medium-flowing; FF = free-flowing; SL = soft lumps.

‡ ND = nondusting; VD = very dusty; FD = fairly dusty.

§ On this product, the laboratory shaking test conclusions were further verified by preparing duplicate packages containing 1, 15, and 50 lb. dry mix, respectively, and duplicate 50 lb. bags of three grades of final feeds representing finer as well as coarser grain feeds. All of these packages were subjected to extensive vibration tests on a machine that simulates truck travel over rough roads. The dry mix was shaken for six hours (equivalent to 3000 miles), and the final feeds were shaken for one hour. Measurements of the dienestrol diacetate concentrations on samples taken from various portions of all the packages indicated that there was no stratification at any time.

TABLE II

Chick Oviduct Screening Tests

DD supplement	DD concentration, m./lb. feed	Chick oviduct Wt., mg.	Chick oviduct Wt., mg. %*
Control			
Test 1	0	13.1	15.9
Test 2	0	10.9	16.3
Test 3	0	8.0	11.5
Test 4	0	10.9	13.6
Oil solution	32	133.9	167.9
Dry mix			
Corn distillers' dried grains	32	130.0	165.6
Milo flour	32	119.8	172.4
Soya flour	32	121.9	172.1

* This means mg. of oviduct per 100 Gm. body weight.

and suitability in actual use. In using the chick oviduct test for such screening purposes, the criterion used is the weight of the oviduct when such chicks are fed the stated amounts of estrogenic substance from the fourth through the eighth day of age. Groups of 15 chicks were used for each given set of conditions, and the results are reported as the group averages. Table II summarizes the results obtained with the preferred dry mix in comparison with dienestrol diacetate oil solution and the untreated controls. The hormonized feed contained 0.007 per cent dienestrol diacetate (32 mg. DD/lb. feed) in all cases. Hormonizing was conducted from the fourth through the eighth day, when birds were slaughtered. Values in the table represent averages of 15 individual chicks. All chicks were from the same hatch of white Leghorn pullets. Since these data indicated that the dry mix was just as effective as the oil solution, it was decided to proceed with the next stage, that is, confirming efficacy of the product.

COMMERCIAL FIELD TRIALS AND BATTERY TRIALS

A commercial grower in Pennsylvania, who had been using the oil suspension previously, was selected for the large-scale field trials. At the same time, it was considered advisable to conduct independent check tests at a College Experiment Station. In order to make certain that the untreated birds were identical in both the field trial and the College trial, it was decided to conduct all the nonhormonized feeding for the first seven weeks at the commercial farm. At the end of this period, the required numbers of birds, about two thirds males and one third females, were carefully transported to the Experiment Station. Application of hormonized feed commenced at the beginning of the eighth week at both locations. In each case there were pens or batteries on feed estrogenized with oil solution, on feed made with dry mix, and on untreated control feed. The detailed conditions of these tests are set forth in figure 1. All birds were grown from the start through 7 weeks at a commercial farm in Pennsylvania during March to May, 1955. Hor-

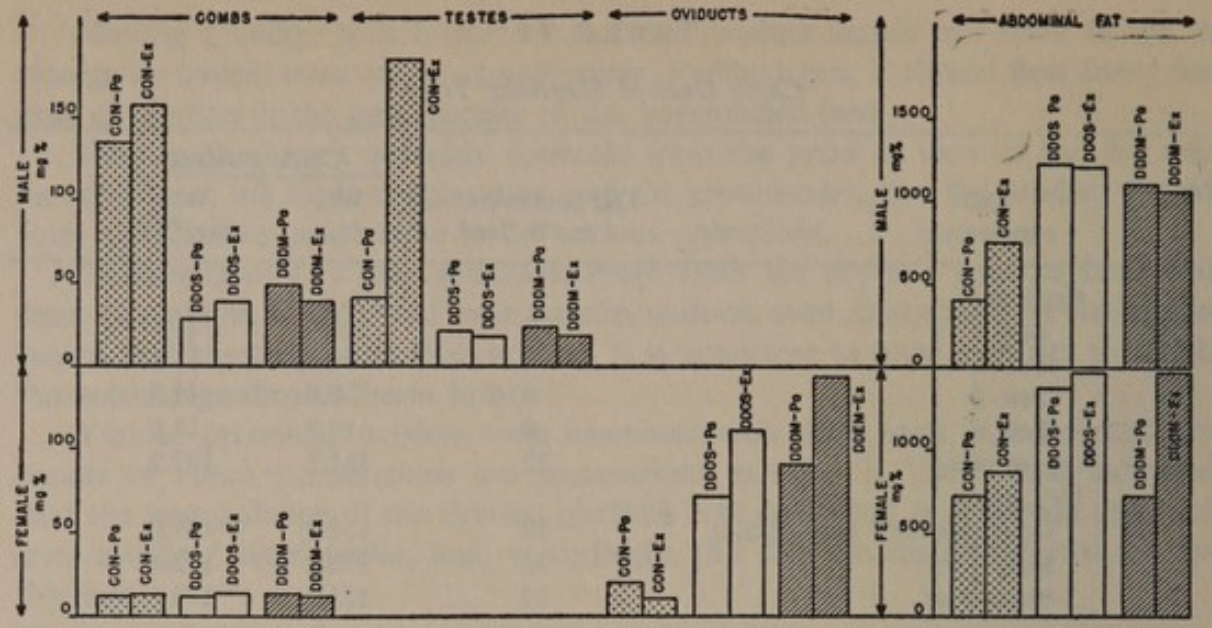

FIG. 1. End organ responses. Con—Pa: controls at the Pennsylvania Farm; Con—Ex: controls at the Experiment Station; DDOS—Pa: oil solution at the Pennsylvania Farm; DDOS—Ex: oil solution at the Experiment Station; DDDM—Pa: dry mix at the Pennsylvania Farm; DDDM—Ex: dry mix at the Experiment Station.

monized birds were finished with dienestrol diacetate oil solution (DDOS)* or alternatively with dienestrol diacetate dry mix (DDDM).* DDOS is marketed as a 7 per cent suspension in soybean oil. Two lb. of this product is dissolved in 18 lb. of hot soybean oil and added to 1 ton of feed. Thus, the final concentration of DD is 0.007 per cent or 32 mg./lb. DDDM is marketed as a 14 per cent mixture with corn distillers' dried grains. One lb. is mixed with 9 lb. of cereal diluent, and this premix is added to each ton of feed, making the same final concentration of 0.007 per cent or 32 mg./lb.

The average feed and DD consumptions for the respective groups were as follows: Three week hormonizing period: 4.5 lb. of feed containing a total of 144 mg. DD; 4 week hormonizing period: 6.7 lb. of feed containing a total of 214 mg. DD. The birds were finished at two locations: at the commercial Pennsylvania farm, approximately 900 birds per pen, and at a College Experiment Station, the birds were transferred at the end of the seventh week for these battery trials, in duplicate groups of approximately 12 birds each, mixed sexes. All the figures on the graph are averages of 10 birds per pen on the commercial field tests and of all the birds in the batteries at the university testing station. The results are expressed in mg. per cent, i.e., mg. of organ per 100 Gm. of body weight.

It has been well established by extensive observation and experience that the end organs provide a reliable basis for evaluating the effectiveness of poultry hormonizing. Thus, parallel series of tests of this type, in which a substantial number of birds are used and in which the weights of these end organs are measured quantitatively, give a reliable basis for comparing the performance of the dry mix with the oil solution. These data are further validated by the independent check results in the battery trials at the Experiment Station as compared with the

* The trade name of White Laboratories, Inc., for the dienestrol diacetate solution and the dienestrol diacetate dry mix is Lipamone.

commercial field trials, in spite of the fact that the latter used a slightly longer hormonizing period. The data indicate clearly that the dry mix is just as effective as the oil solution. Furthermore, these data give convincing evidence of the over-all efficacy of hormonizing birds by this method.

CONTROL ASSAY FOR HORMONIZED FEED

In order to check the quality of the dry mix and the finished feed, a colorimetric assay method has been developed.

The dienestrol diacetate is first extracted with hot chloroform; the extract is filtered, and the chloroform is evaporated from the filtrate. The residue is taken up in ether, the solution is decolorized with carbon black, and the ether is evaporated from the decolorized solution. The residue is then dissolved in glacial acetic acid. A few milliliters of a solution of vanillin in glacial acetic acid are mixed with an aliquot of the glacial acetic acid solution of the extract, and then hydrochloric acid is added. The solution is heated for 15 minutes, cooled quickly, and shaken with chloroform. The absorbance of the acid layer is measured in a colorimeter at 630 $m\mu$ and is compared with that of a standard solution of dienestrol diacetate similarly treated.

The new assay method described represents an improvement over the method currently in use in that this new procedure eliminates the need for using a blank feed with the standard. This improved method is being checked on a variety of feeds before release for general use.

MODIFIED BIOASSAY FOR ESTROGEN RESIDUES IN TISSUES

Since the chemical method is not sensitive enough to detect minute amounts of estrogen, it is necessary to use a bioassay method. The method selected was that of Kahnt and Doisy[1] as modified by Curtis et al.[2] Dehydration of the raw tissues was considered desirable for enhancing lipid extraction. In order to accomplish this without any possible effect on compounds that might have estrogenic properties, lyophilizing was used for this dehydration. The procedure was checked by control samples injected with known amounts of estrogen; tests proved that the recovery of this estrogen was complete.

Specimens were obtained from several parallel-grown dienestrol diacetate treated and control groups (about 25 to 50 birds per group). There were approximately 5 birds in each group sample. The tissues of the birds in each group were separated and were pooled for each group as follows: skin, edible flesh (major bones removed), abdominal fat, and liver.

The skin and flesh were ground to the consistency of hamburger, and then all tissues were dehydrated by lyophilization as previously described. The dried tissues were then subjected to complete extraction of lipid substances by the standard Soxhlet method, using diethyl ether as the solvent. Extract and residue were both saved. The ether was removed from the lipid fraction by evaporation.

Initially, pilot assays, using 5 or 10 rats per group, were carried out. The ether extracts were injected into the test animals, and then the vaginal smears were obtained and read.

A large number of tests conducted in this manner showed no detectable

estrogen in the edible portions of the tissues. Hence, it was considered desirable to improve the sensitivity of the method so that smaller quantities could be detected if present; this would give an even better assurance of safety. This was accomplished by a partial isolation of any active estrogen that might be present in the extracts. By means of this procedure, the sensitivity of the assay was increased substantially; details of this method are to be described in another paper.

The question of the tissue content of conjugated estrogens was also studied. The dried, ether-extracted tissues were subjected to a series of additional extractions designed to remove any conjugated estrogens that might be present. The solvents used included water, acetone, methanol, ethanol, propanol, butanol, and ethylene dichloride. The pooled extracts of each tissue were also assayed, and it was found that no estrogenic activity could be detected.

After the data obtained in these tests were evaluated, it was apparent that the administration of dienestrol diacetate to poultry, at the recommended level, did not result in addition of any deleterious or toxic substance to the edible tissues.

SUMMARY

1. Oral hormonizing of poultry can be accomplished by incorporating in the feed an oil solution of dienestrol diacetate or, alternatively, a suitable dry mix.

2. Equal efficacy for both dosage forms has been verified by chick screening tests followed by large-scale field trials at a commercial farm as well as by battery tests at an agricultural experiment station.

3. Potency and uniformity of feed are readily controlled by a colorimetric assay method developed for the purpose.

4. Safety of the edible tissues for human consumption has been established by extensive tests using the well-known rat vaginal smear assay method that has been modified to obtain substantially improved sensitivity.

5. Experience indicates that the dramatic end organ responses are a reliable indicator of effective hormonizing and that this oral method produces the results without any shock whatsoever to the birds; on the contrary, they give clear evidence of being tranquilized by the transition to hormonized feed.

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Common Sense and Coccidiostats

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The Bible is widely praised as an extraordinary piece of literature because analogy and parable are used throughout. On the other hand, scientific writings are severely criticized as being unreadable and obtuse to the point of incomprehensibility. Although we do not aspire to literary excellence, we may be permitted the use of analogy in attempting to clarify this subject.

AN ANALOGY FROM ENTOMOLOGY

English,[1] in an invitation paper presented at the annual meeting of the Entomological Society of America, discussed "The Need for Common Sense in the Control of Insect Pests." The introductory paragraph of this interesting paper is quoted exactly:

"Several years ago, at one of these meetings, I sat down by one of my old friends from the Cotton Belt, and by way of making polite conversation, I asked, 'Well, how did you make out with the boll weevils this year?' He replied, 'Man, we had plenty of them, but we got wonderful control.' After a moment's pause, he continued, 'But I tell you the bollworms nearly ate us up.' This incident amused me no end, but it is the kind of story that has been repeated time and again during the past few years. The use of chemicals that solve one problem but create another, requiring another chemical which in turn may possibly require still another does not make very good sense, to say the least."

English[1] points out that this trading of one problem for another is by no means limited to the insects affecting cotton. Examples can be cited, *ad nauseum* if not *ad infinitum.*

There are many reasons why the control of one insect by use of chemicals upon plants may be followed by serious outbreaks of another and formerly inconspicuous insect pest. For purposes of analogy we may state that use of an insecticide to destroy one pest alters the environment and may favor survival of another species, which may become a serious pest in its turn. This principle may very well apply to the control of disease-producing organisms among our domesticated fowls and animals.

COCCIDIOSIS AND OTHER DISEASES

The continuous feeding of certain chemicals, collectively known as coccidiostats, has greatly reduced the economic significance of coccidiosis. No doubt the use of coccidiostats must continue for economic reasons. But other diseases are

common during the brooding period. Have losses from these several infections shown any upward or downward trends of significance?

Recently, a feed manufacturer told us, with complete complacency, that coccidiosis is not a problem in flocks financed by his company, but blackhead during 1955 was a disease of major importance. He wanted to add another drug to control blackhead as well as coccidiosis. Is this using one chemical to solve one problem, but create another?

The disease has varied from inquiry to inquiry. Paratyphoid, synovitis, and anemia and its terminal manifestation, hemorrhagic disease, have been mentioned repeatedly. No reliable statistics are available, and we may be unduly impressed by these complaints. Nevertheless, the analogy to the results following use of the newer insecticides is obvious. Are we trading one disease for another? We do not know the answer.

COMPATIBILITY OF NICARBAZIN AND FURAZOLIDONE

Because many individuals wished to use nf-180 (i.e., furazolidone) in feeds containing nicarbazin and an antibiotic, we undertook a small test to determine the compatibility of these drugs. Twenty day-old chicks of male sex and White Leghorn breed were placed in each of 10 pens of an electrically heated, battery brooder. Drugs were administered continuously in the feed. The data are presented in table I where it may be seen that birds receiving 0.011 per cent furazolidone, with or without oxytetracycline, grew approximately as well as those receiving corresponding diets. However, birds receiving 0.0125 per cent nicarbazin were consistently retarded to a degree that proved highly significant by statistical tests. This observation is not contradictory to previous publications, since no observations of the effect of nicarbazin upon disease-free fowls raised on wire-mesh floors have been published, to my knowledge. This test was discontinued when the birds were 4 weeks of age.

TABLE I

Growth of White Leghorn Cockerels on Continuous Medication*

Pen no.	Medication	Mean weight in Gm. 11/8/55	11/22/55	12/6/55
3	Control	39.4	142.3	324.9
9	Control	39.2	137.3	300.8
1	0.0125% nicarbazin	39.4	129.2	277.7
2	0.011% furazolidone + 0.011% oxytetracycline	39.4	143.9	299.1
7	0.011% furazolidone + 0.011% oxytetracycline	39.0	140.2	299.0
4	0.0125% nicarbazin + 0.011% furazolidone	39.3	116.0	254.3
6	0.0125% nicarbazin + 0.011% furazolidone	39.2	130.8	277.0
5	0.0125% nicarbazin + 0.011% furazolidone + 0.011% oxytetracycline	39.5	133.5	286.7
8	0.0125% nicarbazin + 0.011% furazolidone + 0.011% oxytetracycline	39.4	131.2	275.2
10	0.0125% nicarbazin + 0.011% furazolidone + 0.011% oxytetracycline	39.3	136.8	293.2

* The birds were received as day-olds on November 8, 1955, and placed on medicated feed. There were 20 birds per pen.

TABLE II

*Effects of Nicarbazin, Oxytetracycline, and Furazolidone upon Chickens Infected with Blackhead**

Pen No.	Medication in feed	12/6/55	12/21/55	12/27/55	Deaths from blackhead	Note on necropsy
1	0.012% nicarbazin	277.7	445.2	507.2	2	All birds had scars and/or lesions in liver lobes. 7 had impacted cecum.
2	0.011% furazolidone + 0.011% oxytetracyline	299.3	542.8	611.6	1†	Three birds had liver scars. All others were clear.
3	Control	322.0	498.3	576.9	2	Sixteen birds had liver lesions or scars. 15 had impacted cecum.
4	0.0125% nicarbazin + 0.011% furazolidone	254.3	471.6	523.4	0	All livers were clear.

* The White Leghorn Cockerels were 4 weeks old at the time of infection. Each bird received 1000 embryonated Heterakis eggs December 7 and similar numbers December 9 and December 13, 1955. There were 20 birds per pen.

† This bird showed no lesions characteristic of blackhead but died from unknown causes.

Four of the 10 pens detailed in table I were infected with Heterakis on December 7 in a new experiment to ascertain the compatibility of nf-180, oxytetracycline and nicarbazin in the presence of a disease. The test is detailed in table II, where it may be seen that nf-190 (i.e., furazolidone) in the presence of oxytetracycline protected the fowls from blackhead. The effects of nicarbazin upon blackhead and upon the growth of the experimental subjects is apparent at a glance.

Pens 1 and 3 were severely affected by blackhead. Indeed, morbidity in pen 1 was 100 per cent one week after infection, at which time the birds in pen 3 showed only a mild reaction. However, the birds in pen 1 also recovered more rapidly, possibly because of the effects of nicarbazin upon *Histomonas*.

RETENTION OF COCCIDIOSTATS IN THE TISSUES

In a recent paper, Porter and Gilfillan[7] have shown that nicarbazin fed continuously at 0.02 per cent of the diet, accumulates in the liver until 40 ppm are present. Other tissues of the bird show 10 to 12 per cent as much. Yet tissues well removed from the intestinal tract or the liver may exhibit dysfunction when fowls are fed nicarbazin in the diet. McClary[5] states: "Nicarbazin, at a level of 0.0125 percent of the ration completely blocked . . . deposition of the brown pigment ooporphyrin on the egg shell." Ott et al[6] verify this and add that "Hatchability was reduced when Nicarbazin was fed, but returned to normal 2 to 3 weeks after discontinuance of the drug." Our observations are similar. We may add, the shells of eggs laid by birds receiving the drug are rough, fragile, and porous.

Unquestionably, these several observations prove dysfunction of the female reproductive system, which possibly indicates increased susceptibility to disease.

In these respects, the nitrofurans differ greatly from nicarbazin. Investigations carried out at Eaton Laboratories and given to us by letter show that neither

TABLE III

*Effect of Furazolidone, Ntirofurazone, and Arsanilic Acid upon Pullorum and Coccidiosis**

Pen No.	Medicament	Males	Females	Death caused by: Pullorum (number)	Death caused by: Coccidiosis (number)
1	0.0055% furazolidone + 0.0066% arsanilic acid	1053.5	898.0	13	1
2	0.0066% arsanilic acid	900.6	813.7	37	11
3	Control	900.3	745.0	38	15
4	0.0055% nitrofurazone	975.5	812.3	21	3
5	0.0055% nitrofurazone + 0.0066% arsanilic acid	1002.1	788.9	22	1

* The birds were hybrid chickens obtained from a commercial hatchery as day-old chicks on September 25, 1952. They proved to be infected with pullorum as the organism was isolated and identified from chicks dying during the first week of the test. They were vaccinated intranasally against Newcastle as soon as received and placed in five pens each of 150 square feet floor space. There were 200 birds per pen. All birds dying during the test were necropsied to ascertain the most likely cause of death. The feed was of the high energy type without fish solubles, dried whey or extra vitamins.

nitrofurazone or furazolidone can be detected in tissues of fowls given large doses of these drugs although the tests are accurate at one part per million. Williams Smith[8] was able to detect in heavily dosed birds, bactericidal levels of furazolidone that presumably were less than 1 part per million. Francis and Schaffner[2] found that 0.0055 to 0.022 per cent furazolidone or nitrofurazone had no harmful effect on egg production, hatchability, or shell quality when fed to pullets for three months. Our observations are similar although less extensive.

EFFECT OF COCCIDIOSTATS ON INTESTINAL BACTERIA

Certain coccidiostats are effective against coccidiosis only; others affect a variety of diseases. It is well known, for example, that the nitrofurans at 0.011 per cent or more of the diet[3, 4, 8, 9] are effective against the *Salmonella* infections. Lower levels have beneficial effects that have not been so well documented. We were able to observe these effects when we purchased, from a commercial hatchery, 1000 hybrid chicks that were naturally infected with *Salmonella pullorum*. Details of the test are presented in table III. Inspection of the table shows that arsanilic acid, which was used in three of the five pens, had very little effect upon the health of these birds. It may have increased growth rate slightly, but it apparently did not prevent deaths to any significant degree from either pullorum or coccidiosis. Nitrofurazone was used in two of these pens. It reduced the losses from pullorum significantly, but not strikingly. The fifth pen received both furazolidone and arsanilic acid. The deaths from pullorum in this pen were strikingly reduced to approximately one third of those in the pens not receiving a nitrofuran and they were limited to the first week of life. Similarly, deaths from pullorum in the two pens receiving nitrofurazone were limited to the first two weeks of life. On the other hand, deaths from pullorum continued in the control pen for nearly five weeks. The two pens not receiving a nitrofuran and the pen receiving furazolidone were continued on their respective diets until they were in 50 per cent production. Five

and seven-tenths per cent of the pullets receiving furazolidone continuously in the feed were reactors to the usual blood test. On the other hand, 38.9 per cent of the birds receiving arsanilic acid alone and, 41.9 per cent of the controls reacted to the whole blood test. Again we observe that the arsanilic acid had no significant effect. During the first 70 days of production, 87 hens in the pen receiving furazolidone laid 1732 eggs, the 72 hens receiving arsanilic acid laid 755 eggs, and 62 hens receiving no medication laid 902 eggs. Thus the birds receiving furazolidone showed better survival, fewer reactors, laid eggs earlier and laid more eggs than the birds receiving arsanilic acid or no drug in the diet.

Evidence from other tests demonstrates that 0.0055 per cent furazolidone fed continuously in the diet effectively prevents infection with *S. pullorum* under conditions of natural exposure. No guarantee of absolute protection can be given. Indeed, a guarantee of absolute protection against any disease given for any drug is unwarranted exaggeration.

The prophylactic activity of furazolidone is by no means limited to *S. pullorum.* We have some evidence from laboratory or field tests that 0.0055 to 0.011 per cent of the drug may prevent infection with *S. gallinarum, S. typhimurium* as well as other paratyphoids, *Paracolobactrum,* and the mucoid phase of *Escherichia coli.* We lack evidence for *Proteus* only within the important group of the colon-typhoid-dysentery bacteria. We have seen synovitis prevented in large flocks by 0.0055 per cent furazolidone, while similar flocks under the same roof showed a high incidence, although they received nitrophenide and 3-nitro-4-hydroxy phenyl arsonic acid in the diet. At least two coccidiostats in common use today seem to affect bone formation deleteriously and may possibly increase the severity of synovitis epidemics. Higher levels of furazolidone (0.011 per cent) are needed to influence CRD favorably.

DISCUSSION

In order to make the best possible use of the superior prophylactic and therapeutic activity of furazolidone, which is more expensive than some coccidiostats, we have proposed using 0.011 per cent furazolidone in the first 2 lb. of feed each bird consumes. This covers the period when many diseases become established in the poultry flock. Subsequently 0.0055 to 0.008 per cent nitrofurazone is employed to prevent coccidiosis. At times of unusual stress, when the flock is vaccinated for Newcastle disease or bronchitis, for example, 0.011 per cent furazolidone may be used again in the mash for 7 or 10 days. Field reports on the use of this regimen have been most encouraging. A veterinarian who maintains a diagnostic laboratory in Texas for a feed company has reported to us that anemia and its terminal manifestation, hemorrhagic disease, is the number one disease problem in his area; arthritis or synovitis is number two; but in flocks financed by his company which receive furazolidone at two periods, there are no disease problems. Let us emphasize the phrase "no disease problems," does not mean absolutely no disease.

SUMMARY

The recent introduction of highly effective insecticides has proved to be a mixed blessing to farmers. These biologically potent chemicals have altered the environ-

ment of insect pests so extensively that formerly inconspicuous species of insects have appeared in large numbers with disastrous effects.

Coccidiostats are used in a more restricted environment, namely, in the chicken. Nevertheless, by analogy, opportunities for trouble are numerous. Diseases formerly rare, hemorrhagic disease and synovitis, for example, are now frequently reported. Any coccidiostat that deleteriously affects the normal function of the tissues may favor development of a pathologic state. This is particularly true if the coccidiostat accumulates in the tissues in measurable quantities. By using such a drug the poultryman may risk trading one problem for another. Does this make sense? On the other hand, coccidiostats that appear in the tissues at very low levels or not at all, and that possess prophylactic action against a wide variety of disease agents appear less hazardous.

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SYMPOSIUM ON MEDICATED FEEDS

Panel Discussion, January 24, 1956

Moderator: DR. HENRY WELCH

Members of the Panel:

DR. STACY B. RANDLE
DR. MARK WELSH
MR. W. E. GLENNON
DR. COLLINS
MR. R. F. KNEELAND

Symposium on Medicated Feeds

Panel Discussion January 24, 1956

MODERATOR: DR. HENRY WELCH

DR. WELCH (MODERATOR): I have a number of questions that have been proposed by members of the audience. Before we get under way I would like to introduce the members of the panel: Dr. Stacy B. Randle, representing state feed officials; Mr. W. E. Glennon, representing feed manufacturers; Mr. R. F. Kneeland, of the Food and Drug Administration; Dr. Mark Welsh, representing a drug manufacturer; and Dr. Collins, who is in charge of the Veterinary Branch of the Medical Division of the Food and Drug Administration.

I think, perhaps, it would be best to start the panel by having each member of the panel speak briefly concerning the area to be discussed by the panel in his field. I will call first on Dr. Randle.

DR. RANDLE: Thank you Dr. Welch. First, I should like to say that it has been a pleasure for me to be here. I appreciate the opportunity to meet with the group and I feel that bringing all the people together from the various areas represented is a very fine idea to get information assimilated. We can discuss our problems together and, as has been said, "men are never so likely to settle their problems rightly as when they discuss them freely."

DR. WELCH (MODERATOR): Thank you Dr. Randle. Now we shall hear from a feed manufacturer, Mr. Glennon.

MR. GLENNON: I touched briefly on the feed manufacturing industry's interest in this symposium when I spoke a few moments ago. I certainly do think it is a constructive step when members of the Food and Drug Administration, suppliers, the state agencies, and others who are vitally interested in this problem can come together and freely discuss the various aspects that are involved. Medicated feed is a very important part of our industry and the trend, I think, will continue. Certainly, in the area of medicated feed, we are probably more directly concerned than even some of the other groups. Although the responsibility to work on these problems and to solve them to the best of our ability is the responsibility of everyone, I think that if we all work together we can do this.

DR. WELCH (MODERATOR): Thank you Mr. Glennon. I would like now to call on Dr. Mark Welsh, representing a drug manufacturer.

DR. WELSH: It seems to me that the medication of feed has been a rather logical development over the years. I remember some years back, when I was associated with the State of Maryland, that Les Bopst, who was then State Chemist, as he is now, was confronted with the unexpected problem of having vitamins added to commercial poultry feed. This necessitated the development of testing

procedures and standards that at that time were little understood and poorly developed. In fact, there were many people at the time who considered any addition to a natural feed as being an adulterant and therefore illegal and improper.

Since those early beginnings, startling improvements have been made in the nutritive values of feed and highly effective agents have been added for the prevention or treatment of diseases and conditions that lower the quality or increase the cost of the birds or animals being fed. I think the livestock and poultry producers in this country have been most fortunate in having competent and realistically thinking men in State and Federal agencies, as well as in industry, combining their skills and judgment in providing safe and efficient feeds for their use.

I do not remember if it was clearly brought out by any of the speakers during this symposium, but I would like to say it is my belief that there is no nutritional value in the antibiotics that are fed but rather that the beneficial effects are a reflection of the improved health of the birds or animals through the suppression of injurious organisms and toxins. The effects of antibiotics are not confined to the intestines or some other part of the body, but their effects are general in character. One of the reports presented here on the us of penicillin in the diet of chickens indicated as good results in the first 4 to 6 weeks of life as those produced by any of the broad-spectrum antibiotics. I think it would be most desirable that the comparisons be carried on to the usual marketing age of such birds, since there is a question in my mind from the data I have seen that these favorable comparisons would continue.

I have enjoyed attending this symposium and have learned much from this conference. I sincerely hope that it has proved equally profitable to our hosts in the Food and Drug Administration and that they will be kind enough to invite us again.

DR. WELCH (MODERATOR): Thank you Dr. Welsh. I shall call on Mr. Kneeland, a representative of the Food and Drug Administration.

MR. KNEELAND: This symposium has given us an opportunity to bring into sharp focus the manifold problems that the development of medicated feeds has brought on. I do not know that there has been anything startling developed at this meeting, at least to the extent that the problem was unknown before we got here, but I do think we will all benefit by having this opportunity to sit down and view our mutual obligations and responsibilities to the farmer in this very important field. I have a feeling that perhaps tomorrow, personally, I shall feel like I do the morning after having bowled for the first time during the season—a little battered and bruised. I make that remark because I have had the opportunity to see some of these questions and I know some of these questions have been presented before. We will try to answer them, but do not bruise me too badly when you get the answers.

DR. WELCH (MODERATOR): Thank you Mr. Kneeland. I think now we shall start with the first question. "Since roughage quality is the basis for determining how much protein supplement to feed cattle, why then are your stilbestrol tag labeling regulations limited to a flat 'feed 1 lb. per head daily' or 'feed 2 lbs. per head daily,' without any regard to the quality of the roughage being fed?"

We shall start with Mr. Kneeland.

MR. KNEELAND: The questioner fails to recognize that, in acting upon a new

drug application, the problem of safety must be considered in the light of the directions for use, which must be specific. Applications have been made effective for products providing for various levels of diethylstilbestrol per pound of feed. For example, the directions for use may provide for feeding 1, 2, or 4 lb./day/head so that, if followed, the animal will receive, in each instance, 10 mg./day of diethylstilbestrol, the authorized quantity. There is no reason why a protein supplement containing diethylstilbestrol at any of the authorized levels that takes into consideration the roughage quality cannot be selected by the feeder.

DR. WELCH (MODERATOR): There is a second portion to the question. "Objection has been raised by the Food and Drug Administration to supplementary applications for cattle feeds with stilbestrol when feeding directions specify less than 1 lb./head daily. The basis for this objection is that some cattle will eat more and some less. Isn't that true, even when 1 or 2 lb. of supplement are recommended? Cattle coming up to the bunks first get more and those that hang back get less. Why not be concerned with the practical aspects of cattle feeding?"

MR. KNEELAND: I do not know whether the assumption that an objection has been raised to feeding of less than 1 lb. is right. I shall have to consult with my colleague, Dr. Collins.

DR. COLLINS: I think we have been concerned with the practical aspects of cattle feeding. Although we heard a paper by Dr. Gossett yesterday, which stated that doses of diethylstilbestrol up to 50 mg./head/day do not result in any estrogenic activity in any edible tissue, there is no advantage to increasing the amount over 10 mg./head/day. When we voiced our objection to mixing 10 mg. in a quantity of supplement less than 1 lb., we did not have the information furnished by Dr. Gossett, and it is obvious that the more concentrated the diethylstilbestrol becomes in a supplement the more likely it is that a greedy steer will get more than his share. However, we all recognize that, over a feeding period of 100 to 150 days, the average daily consumption of supplement per steer will become more equalized on an individual basis. We still think that there is no valid reason for the 10 mg. dosage of diethystilbestrol to be carried by less than 1 lb. of supplement and we shall request proof for such a need before we give it our sanction.

DR. WELCH: (MODERATOR): The next question is also for Mr. Kneeland. "When a bag of feed is labeled, for example: 'X arsonic acid *mix* for stimulation of growth of broilers' rather than 'broiler Feed containing arsonic acid for growth stimulation,' isn't it actually *less* descriptive of what the bag contains?"

MR. KNEELAND: No.

DR. WELCH (MODERATOR): That is a good answer.

"What is the reaction of Food and Drug officials to Dr. Gibbs' proposal to simplify the labeling of medicated feeds?"

MR. KNEELAND: The problem of simplification of medicated feed labeling has been given continued and extensive consideration. We have not as yet had an opportunity to study Dr. Gibbs' proposals. I am not prepared at this time to comment on them. I might add that, at the present time, we have achieved a reasonable degree of uniformity, which we believe has been helpful to all concerned, and feel that changes in the pattern of labeling should be made only if there are good and sufficient reasons for such changes. Our minds, however, are not closed and Dr. Gibbs' suggestions will be carefully considered.

DR. WELCH (MODERATOR): The next question is one on which I think perhaps

we will have to get an answer from the floor. None of the panel members are in a position to answer it properly, so I am told. The question is really a discussion: "Please discuss the health hazards of manufacturing diethylstilbestrol and of handling it in a feed mixing operation." The second part of the question is: "Is the handling of this drug subject to any government control, either Federal or State? What precautions should be taken?"

I think the answer to the second part of the question is, "No, not to our knowledge is there government, State or Federal, control on the handling of diethylstilbestrol within a manufacturing plant." I would like to ask Brooks Fortune, if he is here, to answer the first part of the question.

DR. FORTUNE: Pure diethylstilbestrol is a dangerous drug and therefore must be handled with extreme precaution, including special ventilation, the use of respirators and of rubber gloves, and the wearing of special clothing. However, when diethylstilbestrol has been diluted in a premix to a concentration of 1 Gm./lb., it ceases to be a dangerous drug and may be handled with ordinary sanitary precautions. We recommend that the mixing operator wash his hands with soap before eating. We also recommend that normal conditions of sanitation and cleanliness should be followed with respect to clothes, that is, work clothes should be changed at least once a week. Other than these two normal conditions of sanitation, no other precautions are necessary in the handling of the premix during the mixing operation.

DR. WELCH (MODERATOR): The next question is really a similar question. "What precautions are recommended for those humans who come in intimate contact with medicated feeds either in manufacturing, mixing, or feeding with respect to (1) respirators, (2) protective clothing, and (3) any other precautions."

DR. FORTUNE: In the mixing or feeding of medicated feeds, there are no special precautions required other than normal and ordinary sanitation, such as washing the hands and an occasional change of clothes, as described under the question of diethylstilbestrol in feeds. However, personnel engaged in working with the pure drugs in the preparation of the premixes should take the normal precaution common to the handling of chemicals, including such special devices as respirators, rubber gloves, and so on. Inasmuch as these are usually handled by trained personnel in plants where chemical manufacturing is commonly carried on, such precautions are routine and commonplace. Beyond the point of the premix as already noted, there is essentially no danger in the handling of the medicated products.

DR. WELCH (MODERATOR): There's a second part to this question. "Can levels (concentrations) of antibiotics in air in feed mills be satisfactorily determined and can safe concentrations be specified?"

DR. FORTUNE: Inasmuch as antibiotics are usually prepared at a concentration of approximately 4 Gm./lb. of premix, which is then mixed into a ton of final feed, the concentration of antibiotics in the final feed is very low, approximating roughly 2 mg./lb. When one attempts to determine quantitatively the concentration of antibiotic in dust from such a feed (when 1 lb. contains only 2 mg.), one finds it very difficult to secure a large enough sample to perform a quantitative determination. We do not know of any method of sampling for this low concentration, which will give us a quantitative estimation of the antibiotic present. Due to the innocuous properties of very low concentrations of antibiotics and the very low concen-

tration of antibiotics in the dust in a mill, we do not know of any data that have ever been collected on which a prediction of a "safe concentration" could be made.

DR. WELCH (MODERATOR): I think Dr. Fortune's answer is relevant. In regard to the second part of the question—"can levels of antibiotics in air be satisfactorily determined and can safe concentrations be specified?"—levels certainly can be determined and I can assure you that in plants where antibiotics are manufactured there are plenty of them floating around in the air. In our own laboratories, it causes a problem. We handle a great many pounds of the various antibiotics daily. We can find penicillin, for example, in low concentrations in the air, but it is present in practically every room or every laboratory that is in use. Incidentally, on two or three floors below our laboratory, we can find small amounts of penicillin floating in the air. It makes it somewhat difficult, sometimes, when you are trying to estimate very small quantities in samples that you have on test. In manufacturing plants they use filtered air and some kind of protective clothing, of course, and it is probably likely that the occasional sensitizations that you see in manufacturing plants are perhaps due as much to breathing in of penicillin or streptomycin. Furthermore, handling of streptomycin may cause these reactions.

The next question is for Dr. Collins: "Why not take the words 'animal feeds' out of the Food, Drug, and Cosmetic Act of 1938 and leave control in the hands of the states?"

DR. COLLINS: The Federal Food, Drug, and Cosmetic Act, which became effective in 1938, does not contain the words "animal feeds." It contains a definition for food that reads: "The term 'food' means (1) articles used for food or drink for man or other animals, (2) chewing gum, and (3) articles used for components of any such article." The United States Congress phrased this definition and only Congress can change it. We, in the Food and Drug Administration, are charged with the enforcement of the law and have no power to change it. I would suggest the author of the question consult with his Congressman.

DR. WELCH (MODERATOR): The next question is for Dr. Welsh. "What effect does the feeding of medicated feeds have on a concurrent vaccination program for poultry."

DR. WELSH: It has no effect so far as we know. As a matter of fact, there is some little evidence that it may enhance the development of immunity.

DR. WELCH (MODERATOR): The next question is for Dr. Collins. "Please list the drugs now used in medicated feeds and indicate which ones are in new drug status."

DR. COLLINS: The following may be in poultry feeds: (1) 0.005 to 0.01 per cent arsanilic acid, sodium arsanilate, or 3-nitro-4-hydroxyphenyl arsonic acid for promoting rapid early growth. (2) Sulfaquinoxaline, 0.0125 to 0.025 per cent for prevention and 0.033 to 0.10 per cent for control of outbreaks of coccidiosis. (3) Nitrophenide for the same purpose, 0.0125 to 0.025 per cent for prevention and 0.05 per cent for control. (4) Nitrofurazone for the same purpose, 0.0056 per cent for prevention and 0.0112 per cent for control. (5) Amino nitrothiazole, 0.05 per cent; 4-nitrophenyl-arsonic acid, 0.025 per cent; and furazolidone, 0.011 per cent, for preventing outbreaks of histomoniasis or blackhead in turkeys; at the 0.10 per cent level, amino nitrothiazole is also used for the control of outbreaks. (6) A mixture of di-N-butyltin dilaurate, 0.07 per cent, nicotine, 0.03 per cent, and

phenothiazine, 0.29 per cent, for removal of tapeworms, large roundworms, and cecal worms. (7) A mixture of nicotine, 0.06 per cent, phenothiazine, 0.60 per cent, and 2,2'dihydroxy-5,5'dichlorodiphenylmethane, 0.28 per cent, for the removal of tapeworms, large roundworms or cecal worms. (8) 0.01 to 0.02 per cent nicarbazin or 0.002 per cent arsenosobenzene for preventing outbreaks of coccidiosis. (9) Furazolidone, 0.0055 per cent, for prevention, in birds older than 2 weeks, of fowl typhoid, pullorum disease, and paratyphoid infection; this drug, in a concentration of 0.011 per cent, is used for prevention of those diseases in birds younger than 2 weeks and for treatment of the diseases in birds of all ages. (10) Di-N-butyltin dilaurate 0.0375 per cent for prevention of coccidiosis and hexamitiasis outbreaks in turkey flocks. (11) High-level concentrations of antibiotics for bluecomb or mud fever, hexamitiasis, sinusitis, low-grade enteric infections, and bacterial associates of chronic respiratory disease. (12) Dienestrol diacetate 0.007 per cent for promoting better distribution of fat.

Swine feeds may contain, in addition to high or low levels of one or more antibiotics, the following: (1) Arsanilic acid or sodium arsanilate, not less than 0.005 per cent and not more than 0.01 per cent; or 3-nitro-4-hydroxyphenyl arsonic acid, not less than 0.0025 per cent and not more than 0.0075 per cent for growth promotion. The higher levels may also be of value in herds affected with *Vibrio* enteritis, which is called "bloody scours" or "swine dysentery." (2) Sodium fluoride in concentrations of from 0.3 to 1.0 per cent, 0.044 per cent cadmium anthranilate, or 0.015 per cent cadmium oxide for anthelmintic (large roundworm removal) purposes. (3) Nitrofurazone (0.056 per cent) for treatment of salmonellosis (bacterial enteritis caused by *Salmonella choleraesuis*).

In addition to low levels of one or more wide-spectrum antibiotics, beef cattle feeds may contain: Diethylstilbestrol in sufficient quantity, when used as directed, to provide 10 mg. of the drug/head/day for accelerating weight gains; and/or phenothiazine in quantities to provide 1 to 2 Gm./head/day for systematic control of certain common intestinal parasites by suppressing their reproductive ability.

Nicarbazin, arsenosobenzene, diethylstilbestrol, and dienestrol diacetate are still in a new drug status.

DR. WELCH (MODERATOR): The next question is for Dr. Randle. "You stated yesterday that active drug concentrations in medicated feeds should be within plus or minus 10 per cent of the stated potency based on a composite sample. What variation between random single spot samples is considered satisfactory?"

DR. RANDLE: I cannot answer this because it is a matter that will have to be decided by the individual state. It is my opinion that uniformity of mix is much more important in feeds containing drugs than in those without drugs because of the possible toxicity of an overdose of a drug. In some instances, the animal is much more sensitive to the drugs than is the analytic method.

DR. WELCH (MODERATOR): The next question is for Dr. Collins. "As evidenced by commercial products being sold, there seems to be considerable difference of opinion regarding dosages of piperazine compounds to be used in worming swine. The same is true with dosages for poultry. Would the panel comment on possibilities for standardization of dosage recommendations for use of piperazine salts with both species?"

DR. COLLINS: I am glad that the question reads "would the panel comment on the possibilities."

DR. WELCH (MODERATOR): You are representing the panel.

DR. COLLINS: Since there are no new drug applications effective for the use of piperazine compounds ready-mixed in a manufactured animal feed, there may be some doubt about the propriety of this question at a meeting of this nature. However, it is true that considerable confusion exists concerning what might be termed "the proper minimal effective dose" of piperazine for all animals. When piperazine and its compounds first started to take the animal-production field by storm, the only basis for recommended dosages was laid in reports by British workers. Gradually, we are accumulating more information based on work being done in this country. The situation will probably be chaotic for some time to come. Actually, it is not the responsibility of the Food and Drug Administration to standardize dosage. It is the exclusive responsibility of each manufacturer to assure himself that his products are effective in fulfilling their intended function when used as directed. With respect to piperazine compounds, as with other anthelmintic drugs, the only way to be certain about the efficacy of any given dosage is to support the recommendations by carefully controlled critical scientific tests. However, since all piperazine compounds depend on their piperazine content for anthelmintic efficacy, we have consistently expressed the opinion that the active ingredient of such preparations should be declared as "piperazine (as the .....) ....., (per cent, grams, milligrams,) per ..... (unit of weight or measure) .....," the blanks being filled in with the correct figures or words.

DR. WELCH (MODERATOR): The next question is for Mr. Glennon. "Does the Feed Manufacturers Association feel that their members are properly informed about State and Federal legal requirements?"

MR. GLENNON: Generally speaking, probably not and I think that is a weakness of human nature. I do not think there are very many people who know as much about as many laws as they should. We do try to make an effort in this field to advise our membership of both Federal and State legislation that is of importance to them and I shall admit that we probably do not do enough of it. It is an increasingly difficult job to screen all the Federal and State legislation that is directly concerned with the feed manufacturing industry. There is room for improvement both on the part of the Association and on the part of its members, I would say, in regard to this problem.

DR. WELCH (MODERATOR): The next question is for Dr. Collins. "Toxic levels of medications in feed can and will occur, the human factor being what it is. Why does not the Food and Drug Administration insist that information on the symptoms and lesions produced by toxic levels of medicants be made available to interested persons, particularly the farmer and veterinarians?"

DR. COLLINS: The questioner apparently fails to recognize that the manufacturer of a new drug in current use has proved the lack of toxicity of his product when used as *directed*. No law enforcement agency can protect the person who chooses to ignore the provisions adopted for his protection. The Administration can no more protect the man who ignores directions and warnings than the State police can prevent the injury or death of the man who ignores road signs and other devices provided for his safety. The law requires adequate directions and adequate warnings. To a person of less than average intelligence "use only as directed" should constitute sufficient warning if the directions are adequate. With his background of education and training in medicine and toxicology, the veterinarian

should not have to be informed by the label of a "medicated feed" about the pharmacology and toxicology of the declared active drug ingredient. If he is not currently informed with respect to an unfamiliar drug, he has only to pick up his telephone and call the firm who investigated and made the drug. Certainly the law does not require dissemination of such information.

DR. WELCH (MODERATOR): I had hopes that we would be able to close the symposium by 4:30 and I think we are going to come very close to it. The last question or two questions are for Mr. Kneeland.

"Why can't the feed industry be permitted to make the brand name predominate when the manufacturer of human medicine and food can make the brand name predominate?—Anacin, for example."

MR. KNEELAND: I do not think there is any comparison between the two situations. A better analogy would be the addition of aspirin to bread, which would result in an adulterated food. We have discussed this matter many times and I do not believe that this is the time to review the many factors that have been considered in the development of the present recommendations for labeling medicated feeds.

DR. WELCH (MODERATOR): And now the last question. "If the trade name is in the same size of type as the long chemical name, how do you suggest feed be identified to facilitate handling and avoid a mix-up by laborers on the farm who do not understand the chemical names?"

MR. KNEELAND: If a person cannot read, I do not know how labeling that will be informative can be devised. On the other hand, I do not believe that it is necessary for a laborer to understand the meaning of a chemical or drug name in order to distinguish between a tag label that bears such a name and one that does not. I do not think that the answer to the problem is greater emphasis of the trade name.

DR. WELCH (MODERATOR): That completes the questions that were presented to the panel. It's now about 4:30 and, before closing the symposium, I think we should all thank Dr. Charles Durbin who has been the spark plug behind the actual development and organization of this, to my mind, excellent conference. I want to thank the members of the panel for helping us out this afternoon. The symposium is now adjourned.

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