Essay on Biochemical Genetics

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An introduction to genetical biochemistry.

By J. B. S. Haldane F.R.S.
Weldon Professor of Biometry
University College, London.

Pontecorvo, G. (with sections by J. A. Roper, L. M. Hemmons, K. D. Macdonald, and A. W. J. Bufton (1952). Advances in Genetics.

Biochemical Genetics

Preface (see vol 2)

This book is emphatically not a textbook. It is intended to summarise some of the main facts in a ~~field~~ ~~which is growing~~ branch of science which is growing so quickly that, even if the book had been up-to-date when written, it would have been out-of-date by the time of publication. I have chosen my examples from certain fields of work, and doubtless neglected others which may prove to be more important. The only alternative would have been to fill several volumes. Nevertheless there may be some advantage in trying to cover the whole field of biochemical genetics of unicellular organisms, fungi, higher plants, and animals including man in a single volume. ~~In each~~ Each group is well adapted for the study of some topics, and problems, and ill-adapted for that of others. Thus a bird's-eye view may gain in extent what it loses in precision. We hope

The book is based on lectures given in 1950 and 1951 in the Department of Biochemistry, University College, London. I have to thank many colleagues for help, but particularly Dr. H Harris, who has allowed me to read his book "An Introduction to Human Biochemical Genetics" in typescript and Dr. G. Pontecorvo who has been equally courteous with "The Genetics of Aspergillus nidulans".

However for the benefit of readers who are geneticists rather than biochemists I have given a few formulae in the Appendix.

(and even the beginnings of the adaptive process)

and adaptations are carried out or lost in a small fraction of the life cycle.

Chapter 1.

The elements of genetics

This book is written for biochemists rather than for geneticists. I therefore begin by explaining some elementary genetical notions. I shall assume an elementary knowledge of biochemistry, for example the structural formulae of the amino-acids. Genetics is concerned with differences between similar organisms, and mainly with those differences which are not due to causes acting during the lifetime of the organisms concerned. This distinction works fairly well for higher organisms, but breaks down completely for unicellular organisms. If a cell can divide once an hour, but takes a day to adapt itself to ferment a new type of sugar to which it is unaccustomed, it could a growing population can only adapt if the adaptation is inherited. In such cases special experiments are needed to distinguish between the effects of adaptation and selection. On the other hand there is much less carry-over of this kind in higher organisms where only a very small fraction of the matter in any individual is derived from its parents. An example will show the kind of distinction which we can make in higher Metazoa. A straight-legged bitch produces a puppy with bent legs. This may happen for at least five reasons. The puppy may have been short of vitamin D, and developed rickets, or its father may have been a dachshund. If the father was bad bent legs from rickets, this appears to have no effect on his progeny. If the mother was rickety, rickets may appear in her offspring because of the blood with which she perfused the

all or almost all bacteria,

A Any culture of a haploid organism, such as many fungi, is necessarily a clone unless conjugation occurs within it, and the haploids formed from such a conjugate normally have the characters of the original clone.

Bacteria

foetal placenta or the milk which she gave to the suckling was short of vitamin D. It is particularly hard to draw a dividing line when we are dealing with virus infections.

If we consider any quantitative character, for example the height of a man, the milk yield of a cow during her first lactation, or the sucrose content of a sugar beet, we shall generally find that it is affected by differences both of what Galton called Nature and what he called Nurture. Roughly speaking, Nature covers all causes acting before fertilization, and nurture all causes acting after it. In such a case we find our analysis much easier if we can keep one variable nearly constant, while allowing the other to vary, as when we deduce Boyle's and Charles' laws by keeping first the temperature and then the pressure of a gas constant. We keep the nurture of a group of organisms constant by supplying with them all the same food, water, light, infections, and so on.

There are three different ways of obtaining a population with nearly constant nature (apart from sex differences and the corresponding differences in self-sterile hermaphrodites). The three types of population are:-

1. A Clone, that is to say a population derived from a single cell by a series of mitotic divisions; for example a named variety of apple or potato, a group of aphids derived from one mother by parthenogenesis, or a pair of human monozygotic twins.

2. A Pure Line, that is to say a population derived from a single individual or pair by prolonged self-fertilization or by other-sister mating. Examples are named varieties of wheat or bean, and a few lines of mice, guinea-pigs,

One self-fertilization of a haploid, e.g. a fern prothallium, will give a pure line

The reasons for the genetical homogeneity of such stocks are given in any textbook of genetics.

or group of characters

and Drosophila.

3. The first cross (but not later generations) between two pure lines.

The proof that they are genetically homogeneous is that selection within them is ineffective. You will readily get heavy or light mice by breeding for an the heaviest members of a ~~pure line~~ mixed stock. You will not do so by selecting within a pure line. Nevertheless complete genetical homogeneity is an unattainable ideal like absolute purity, if only as a result of mutation; and pure lines are apt to be weak and sterile. A pure line of cows would be as useless for ~~practical~~ most practical purposes as a bar of pure iron. Both could be very useful for research.

But apart from pure lines we can readily get stocks which breed true for a particular character, say long-haired blue rats, or peas containing starch-grains instead of sugar, and with white instead of purple flowers. Genetics is based on the experimental crossing of such stocks and the analysis of later generations. Geneticists naturally tend to choose for their studies characters which are little affected by the environment. This is an inevitable ~~astic~~ choice by chemists for their preliminary studies of stable inorganic substances rather than metastable organic compounds, or free atoms or radicals. But it is unfortunate for two reasons. Economically important innate characters are generally capacities for response to suitable environments. A good dairy cow is one which produces a lot of milk on an English meadow, but will not do so on a Welsh mountain. ~~Or India~~ either. The mountain cow is less variable in her response. Secondly we can learn a great deal about physiological genetics by altering the environment, for example by showing that a particular ~~strain~~ line of yeast will only

divide when grown in presence of thiamine, or can ferment glucose but not maltose.

Nevertheless we begin with characters little affected by the environment. The sort of result which we get depends on whether we study characters manifested in haploids or diploids, that is to say organisms with one or two sets of chromosomes per cell. I shall not consider the complications introduced by polyploidy, although this is by no means confined to higher plants, as is sometimes supposed. Many normal mammals, including men, have tetraploid liver cells.

In most organisms with sexual reproduction there is a haploid phase with n chromosomes A B C D . . . . Two haploid gametes fuse to give a diploid phase with 2n chromosomes A A, B B, C C, D D, . . . , though sometimes sex is determined by a difference in one chromosome pair. In some organisms such as Ascomycetes the diploid phase only lasts for a short time, and we usually study haploids. In others such as most higher animals or plants the haploid phase lasts for a short time. We do not study differences between spermatozoa, or eggs after the extrusion of the second polar body, though we can do a little with pollen grains and tubes. In other organisms, such as yeasts, seaweeds, mosses, and bees, we can study both haploids and diploids. We sometimes, particularly in plants, find a character which is cytoplasmically determined (cf Chap 7) but such characters are not very common, and in most organisms nuclear determination is much more important. That is why I have emphasized the chromosomal constitution.

Consider an organism in which the haploid phase is studied, such as one

of the mould species N. crassa and N. sitophila, & their hybrids. On crossing a normal (P) and a pale (p) wild stock, we can isolate single asci, dissect them, and grow the 8 spores in an ascus, and note the colour of the 8 haploid cultures so produced. Here the first two divisions are meiotic, the third mitotic. The spores are arranged in a row in the ascus, and we get such orders as PPppPPpp or pppp PPPP, but P and p only segregate at the first two divisions, and there are almost always just 4 P and 4 p spores. About once in 500 asci something goes wrong with the meiosis, and we get less than 8, or unequal numbers. We say that P and p are genes, that is to say structures which are reproduced (or copied) at each mitotic nuclear division, and which segregate regularly in the meiosis of heterozygous diploids. A segregating diploid such as Pp is called a heterozygote, a non-segregating diploid such as PP or pp a homozygote.

If we study the diploid phase we soon find that reciprocal crosses (♀ A × ♂ B and ♀ B × ♂ A) usually give the same result, which at once suggests nuclear rather than cytoplasmic determination. Sometimes the heterozygote is intermediate between the two homozygotes. For example in Primula sinensis a pale pink-centred white form ("Duchess") and a dark red ("Crimson King") breed true. On crossing them we get a pinkish flower ("General Buller"). If the first is called DD and the second dd we have the situation shown in Table 1.

de Winton, D., and Haldane J. B. S. (1933) ~~Journ. Gen.~~ [? of your letter]

^single anther,

*[Footnote] Dominant genes are commonly denoted by ~~two~~ capital letters, recessives by small letters. Sometimes however the "wild type" gene is denoted by +, the mutant gene by a letter. Thus AA, Aa, and aa cases can be denoted by $\frac{+}{+}$, $\frac{+}{a}$, and $\frac{a}{a}$.

Table 1

Parents	Gametes	Zygotes DD	Dd	dd
DD x DD	D x D	DD		
dd x dd	d x d			dd
DD x Dd (or Dd x DD)	D x (D+d)	½ DD	½ Dd	
dd x Dd (or Dd x dd)	d x (D+d)		½ Dd	½ dd
Dd x Dd	(D+d) x (D+d)	¼ DD	½ Dd	¼ dd

There was a fairly good approximation to the expected ratios, but exactly they are rarely fitted exactly, for three reasons. First, we cannot in practice use all the pollen grains produced by a plant, as we can sometimes germinate all the spores in an ascus. And we can only use one of the four haploid cells produced in a female meiosis. So our numbers are subject to sampling error. Second, there is some natural selection, both between pollen tubes and among young plants, favouring one type at the expense of another. Third, mutations occur, though we might have to grow several million plants before we found one affecting the gene in question.

Very commonly we find dominance. If we cross a homozygous tabby (AA)* with a black (aa) cat, all the kittens are tabby (Aa). Tabby is said to be dominant to black, and black recessive to tabby. Heterozygous (Aa) tabbies give equal numbers of Aa and aa when crossed to black, and 1 AA : 2 Aa : 1 aa, or 3 tabby to one black, when mated together. In other words we cannot distinguish between AA and Aa cats. One A gene can do the work of two. It is clear that recessives will breed true, while dominants may or may not do so.

However dominance is a subjective matter. A suitable test may

Sanwal and Glick (1963) Proc Nat. Ac. Sci 29, 55

nearly or quite

distinguish the two kinds of dominant. Sawin and Glick (1943) found that while the serum of most rabbits contain an atropine-esterase, some lack it. When they estimated the amount of the enzyme in the serum they found

In 4 homozygotes, 232-348 units, average 291
In 25 heterozygotes, 52-194 " " 107.

It appears that the homozygotes make about twice as much enzyme as the heterozygote and that in fact the recessive gene is inactive, and each dominant acts independently. It is not very rare to find that ~~a han~~ colour develops rather quicker in a homozygous than a heterozygous dominant, even though the final pseudo stages of development are indistinguishable. This can only happen if the gene in question controls one of the slowest stages in the biochemical process concerned, and thus acts as a limiting factor. It is often, but by no means always, the case that genes normally found in a species (often called wild type genes) are dominant over those which arise by mutation (mutant genes). ~~By~~ In Drosophila it is sometimes possible to introduce more than two of these recessive mutants genes into a single cell by the use of chromosome fragments. Sometimes three or four have no more effect than two, in which case it is concluded that the recessive gene is inactive. Sometimes they produce a fly more like the wild type, and it is concluded that they are performing the function of the normal gene, but less intensely. In such cases dominance simply means that the normal gene has a factor of safety of 2 or more. As just as a man can in most circumstances get a perfectly well with only one kidney or adrenal, and a blood analysis would not reveal the absence of the other.

When a double heterozygote segregates for two pairs of genes

they usually segregate independently. Thus on crossing a homozygous coloured short-haired rabbit (C C L L) with a white long-haired (c c l l) we get C c L l (coloured short-haired) progeny. On crossing these to c c l l we get ~~of~~ about equal numbers of C c L l, C c l l, c c L l, and c c l l, showing that C L, C l, c L and c l gametes are formed in equal numbers.

Fat colour in rabbits is also genetically determined. Most rabbits have white fat. Others have yellow fat ~~provided~~ provided they are fed on green leaves or other food containing ~~xanthro~~ xanthophyll, but not if they are given a diet lacking xanthophyll, such as turnips. These rabbits lack a xanthophyll oxidase present in the normal animals. And provided the diet contains xanthophyll, yellow fat is a good recessive, due to a gene x. If we cross homozygous coloured white-fatted (C C X X) rabbits with white yellow-fatted (c c x x) the progeny are coloured white fatted (C c X x) double heterozygotes. Crossed with c c x x they gave about

86 C X : 14 C x : 14 c X : 86 c x.

Such heterozygotes are called $\frac{C\ X}{c\ x}$ or $\frac{+\ +}{c\ x}$. If we make up C c X x rabbits from the cross C C x x × c c X X we get $\frac{C\ x}{c\ X}$ or $+c\ x\ \frac{+\ x}{c\ +}$ double heterozygotes. Mated to c c x x they gave about:

14 C X : 86 C x : 86 c X : 14 c x.

This kind of geometrical isomerism is called linkage. It has been shown in several animals and plants to be due to the position of the genes on the chromosomes. Genes which are near together tend to be strongly linked. The simplest proof of this arises from the fact that a few genes are visible, some 4 mutants being

Lea, D.E. (1946) Actions of radiations on living cells. Cambridge

differences of a small section of a chromosome, others heavy duplications. They are found where they should be found according to maps constructed on the basis of linkage data. Most genes are however, invisible with a microscope, though the electron microscope may yet reveal some of them.

Genes are generally reproduced accurately at a nuclear division, and that is to say an A gives two A's, and an a two a's. If the accuracy were not very great, selection would be effective within pure lines. ~~Sometimes however a gene is~~ If ~~there were always the case~~ it were always accurate a species could only try out combinations of pre-existing genes, and ~~mutations would be~~ evolution could not have occurred. ~~But~~ In fact genes do not always reproduce their like. The process of ~~change~~ alteration is called mutation It may be due to an alteration in a gene between nuclear divisions, or to an incorrect copying. It occurs with a frequency which is often of the order of 10^{-6} to 10^{-8} per cell division, ~~but~~ may be as high as 10^{-3}. It is worth remembering that a man has about 2^{48} cells, a Drosophila about 2^{24}, so ~~the number of cells~~ so that there are 48 or 24 divisions between an egg and an average somatic cell. The number of cell divisions in the malegerm line is some-what more than this, in the female less somewhat less, so the mutation rate per generation is often of the order of 10^{-5} or 10^{-6}.

We ~~have then a reason~~ can then for our working hypothesis that a gene is an organic ~~site~~ in a particular ~~part~~ region on a particular chromosome, with a definite function, and reproduced with great, but not complete, accuracy, at each cell division. Experiments where mutation is provoked by X-rays (cf Lea 1946) ~~show~~ a method which gives molecular weights of the

or a diameter of ~~4~~ to 40-100 A° (cf Haldane 1920)

weight order when applied to enzymes) strongly suggest that genes commonly have a molecular weight of the order of 10^6. The dimensions of the chromosomes, and the probable number of genes in them, suggest a similar figure.

We can now ask four questions concerning genes. The rest of this book is an attempt to answer them. It will be seen to be a very inadequate attempt.

1. What do genes do? That is to say what are the differences in biochemistry, in physiology, in development, in behaviour, between organisms with different sets of genes? This is a question analogous to that asked by physiologists concerning the function of an organ. We can state the function of the heart or kidney with great accuracy without knowing how they are performed. For example we need not know whether adenosine-triphosphate is concerned in contraction or relaxation, whether sugar is reabsorbed by the tubules by the same mechanism as potassium. But an answer at this level would give us a new cellular physiology and biochemistry, a new embryology, a new psychology, and a new theory of evolution.

2. How do genes do what they do? Does each gene synthesize a specific substance, for example an antigen or an enzyme? Can genes be regarded as enzymes bound to the cell structure?

3. What are genes biochemically? Are they perhaps ~~the~~ desoxy-ribo-nucleoproteins as all enzymes seem to be proteins?

4. How are genes reproduced, and how is their process of reproduction sometimes altered so as to give a gene of a new type? Are such changes bound up

Haldane J.B.S. (1920) Trans. Oxford Univ. Junior Scientific Club 3rd Series 1.3

*[Footnote] As this reference is quite unknown, I may perhaps be permitted to quote the relevant sentence: "The precise nature of their activity is uncertain, but in some cases we have very strong evidence that they [genes] produce definite quantities of enzymes, and that members of a series of multiple allelomorphs produce the same enzyme in different quantities." The same number is more interesting as containing a paper by Soddy on radioactivity. Had its last paragraph been accepted by any government, world history would have been very different.

Schrödinger E. (1944.) What is life. Cambridge

Lysenko T.D. (1944) in "The Situation in biological science" Moscow, 1949

To doubt their cogency

with their functional activity?

It is possible that I am responsible (Haldane 1920)* for the suggested answer to question (4), namely that a gene makes a particular chemical species of enzyme or antigen, though I do not see the idea in Cuénot (1903) nor (Vol 2) in Lotsy. If this answer is correct, which I doubt, it would not of course follow that all antigens or all enzymes were direct gene products. However I doubt if the answer is correct. It is probably true sometimes, and is a useful working hypothesis because it is a guide to experiment, and a sufficiently clear statement to be capable of disproof if untrue.

Two simple answers to question (3) are possible. One is that genes are nucleoproteins like molecular viruses, copied by the rest of the cell by the same (unknown) process. The other is that they are similar to their primary product: the copying process is going on all the time, and most of the copies diffuse out into the cytoplasm; one in each cell cycle is anchored to a chromosome. This has the merit of reducing the number of genic properties requiring explanation, but is probably incorrect.

If one is convinced by the arguments of Schrödinger (14), as I am not, the answer to (4) is the answer to "What is life?" If one is convinced by those of Lysenko (1948), as again I am not, mutation is a highly adaptive process. That is not to say that it is never adaptive. At the present moment geneticists, across Iron Curtain, are well likely to obtain promotion or at least escape dismissal, either by saying "Lysenko is always right" or "Lysenko is always wrong." The history of science renders it highly improbable that either of these statements is correct.

Haldane J.B.S. (1940) New Paths in Genetics. London

due to a rearrangement

Grunberg H (1941) ~~The Genetics of the mouse.~~ Animal genetics and medicine. London.

Before we try to answer even the first question we must say a little more about general genetics. In the first few years of Mendelism people wrote of genes for unit characters. E.g. the presence of hooves in Matthiola incana is dominant over their absence. So authors wrote of hoarseness as a unit character due to a gene H. But it soon turned out that there were several different recessive hoarseless forms. $H_1 H_1 h_2 h_2$ is hoarseless (glabrous). So is $h_1 h_1 H_2 H_2$. On crossing weight $H_1 h_1 H_2 h_2$, which is hoary, and gives 7/16 hoarseless offspring. Further work showed that one of the genes needed for anthocyan production was also needed for hair formation. One cannot speak of a unit character controlled by H_1 and another by H_2. Recently however a series of unit characters in the old sense has been discovered. These are the antigens described in the next chapter.

The next hypothesis was that each gene controlled a unit process (Haldane 1940) presumably capable of description in biochemical terms, which might however play a part in several developmental processes. We shall see that this process, e.g. a particular dehydrogenation, is sometimes known. In other cases it is not. Thus white cats are generally deaf. Mice with a particular type of microcytic anaemia usually have a flexed tail and a white spot on the belly. It is anybody's guess what is the common link in the three developmental processes concerned. Such action of a single gene on very different characters is called pleiotropism. Grüneberg (1941) maintains that there must be a common biochemical process. It is perhaps not inconceivable that a single gene may have functions as different as the secretory and storage functions of the liver, or the antidiuretic and uterine stimulating functions of the posterior pituitary. But Grüneberg is probably correct.

Through chlorophyll-less mutants of green plants will often live on sugar solution.

These are called saprotrophs, and

cock.

for a particular man A   form

Sometimes we may hit on a rabbit which produces very little on the human outer body except on the M or under N.

Chapter 2.

Possible Primary products of gene action.

~~The simplest of all~~ Of all the characters which have been studied, those whose genetics are simplest are the antigens for which ~~most~~ many, and perhaps all species of mammals and birds are polymorphic. We will begin with a very simple example. If one inject human blood corpuscles into a rabbit, the rabbit develops antibodies, and its serum will agglutinate all human blood corpuscles. If we exhaust it with the corpuscles of A, that is to say make it agglutinate several batches of them, it will finally lose all its anti-human agglutinins. But if we exhaust it with the corpuscles of another man B, it will finally cease to agglutinate them, but may still agglutinate those of A. In this way we can produce a rabbit serum which agglutinates the corpuscles of about $\frac{3}{4}$ of the human race, who are said to carry the agglutinogen M. Similarly an agglutinogen N can be detected. These agglutinogens are determined by two allelomorphic genes L^M and L^N. Every human gamete carries one or other of them. All human beings are ~~is~~ of the genotype

$L^M L^M$, with the M agglutinogen on the corpuscles,

$L^M L^N$, " " M and N agglutinogens on the corpuscles,

or $L^N L^N$, " " N agglutinogen on the corpuscles.

Thus none can have the M agglutinogen unless one or both parents had it, and similarly with the N. Further no treatment has ever led to the appearance or disappearance of either of these substances. They appear to depend wholly on nature, and not at all on nurture. No nurtural difference will abolish

for characters others than antigens.

Todd C. PRSB 104, 194. (Callus domesticus)

either of them without killing the person concerned. Further since there is no case not explicable by illegitimacy where two parents without M produced a child with it, one can say with great confidence that only a gene is concerned. This is not generally the case. The compresence of several genes is needed to produce colour in a mouse hair or a Drosophila eye, to produce a full coat of hair on a mouse or wings (other than barely visible ~~rudiments~~ vestiges) on a Drosophila, and so on. Where only one such gene is known to be needed in a species, we have good reason to believe that further research would ~~have~~ reveal others (Thus two genes are needed for anthocyan production in Lathyrus odoratus, so far only one in the related Pisum sativum).

What is more than is a very general (though not quite universal) property of corpuscular antigens. Todd (1930) ~~has~~ immunized one fowl with the blood of a large number of others. ~~He found no case~~ Its serum would agglutinate the corpuscles of all other fowls. If exhausted with the corpuscles of A it would still agglutinate those of B, C, etc., even if they were brothers and sisters. But if exhausted with the corpuscles of any cock and hen, it would not agglutinate the corpuscles of any of their progeny. Thus no fowl has corpuscular antigens not found on the corpuscles of one of its parents. The same holds (so far with one exception) for human beings. ~~Thus~~ Since a great many different antigens are known, each determined by a single gene, this means that each is determined by that one gene, and not (like an anthocyanin or a melanin) by the interaction of several genes. This conclusion is probably false in the ~~for~~ last sense. If an antigen is made in part, say, for or galactose molecule, it requires its own special gene, and also genes concerned in making galactose

On ~~the other hand~~ the amount of antigen in both M and N antigens in heterozygotes is about half what it is in homozygotes, ~~as if the two allelomorphs were acting independently~~ which could be true either if the two allelomorphs were acting independently, or if they were competing for a limited amount of substrate or of space.

Irwin, (19) Advances in Genetics 1,

from other sugars. But these latter are presumably vital, in the sense that their absence is incompatable with life.

~~Let us now consider some exceptions to Todd's law. Some human beings have an~~

However even when antigens obey Todd's law this doesn't mean that they do all the genes are acting independently. For example in members of the human group AB which carry both the A and B antigens on the corpuscles, the amount of A is often a good deal less than in people who have one A gene and one recessive (O) gene. This may be due to competition either for substrate ~~a~~ from which to synthesize the haptens, or for proteins ~~to which~~ to which to attach them. Similarly there ~~are~~ is a good deal more of the H antigen in group O than in other groups. It seems that there is competition between the A and B genes and some other "organ", probably not the recessive O gene. I

Let us now consider some exceptions to Todd's law. The Lewis[a] and Lewis[b] antigens are due to allelomorphic genes. ~~A~~ There is ~~a~~ perhaps a rare third gene ~~being~~ giving Lewis[c]. Homozygotes for the Le[a] gene react with anti-Le[a] throughout life. Heterozygotes only do so in the first 18 months or so of life. Such competition between allelomorphs or antimorphs is known in other fields of genetics. To get the Le[a] antigen in an adult one must have two like Le[a] genes. There is no question of two unlike genes cooperating to make it.

On the other hand I ravin ( ) found that hybrids between several species of dove and pigeon had corpuscular antigens which were not present in either parent species, along with all the antigens of both parents. Of course the genes were in a very abnormal environment. But ~~this~~ This observation is a conclusive disproof of the "~~One gene~~ hypothesis that a particular type of gene

Morgan W. T. J. (1950). Nature 166, 300

Annison, E. F, and Morgan W T J (1952). Biochem. Journ. 50, 460

about 250,000

[The H substance seems to be present on almost all red corpuscles, but there is more of it on those of group O. It is perhaps "crowded off" by the A and B substances).

Grubb R (1948). Nature 162, 933.

always makes a particular type of antigen, and that no antigens are made otherwise. Nevertheless this hypothesis is sufficiently often true to be of value in prediction.

The chemical nature of some of the genetically determined antigens is known {Morgan (1950) }, Jonsson & Morgan (1952) give numerous references}.

The specific components (haptens) are mucoids ~~of~~ high molecular weight which can be obtained from ovarian cysts in large quantities of the order of 300 gms. They differ in their optical rotatory powers The following are examples

	H	A (? A_1)	Le^a
α_D	-35°	+15°	-45°

They seem to have be built up of four sugars, namely D-galactose, L-fucose, ~~D-glucosamine, and D-chondrosamine (the two latter probably as~~ N-acetyl-D-glucosamine, and N-acetyl-D-chondrosamine, and eleven amino-acids namely glycine, alanine, serine, threonine, valine, leucine, proline, aspartic and glutamic acids, lysine, and arginine. L-fucose (6-desoxy-L-galactose) has also been found in seaweeds and the jelly of sea urchin eggs. The percentages found are different, for example A substance contains 18% fucose, Le^a only 13%; A contains about equal amounts of glucosamine and chondrosamine, Le^a about three times as much of the former. Threonine appears to be the commonest amino-acid in each. ~~Unfortunately~~ It is greatly to be hoped that comparative biochemical work may be done on the products of two allelomorphic genes such as A_1 I^A and I^B or Le^a and Le^b.

Le^a has a very remarkable biological property, Grubb (1948) found that Le^a Le^a individuals ~~contain~~ produce saliva and other secretions containing the Le^a substance and so

Further in the secretors the A, B, and H substances are present in a water-soluble form, whilst in the non-secretors they are only present in an alcohol-soluble form, as on the corpuscles. Morgan (Freudenreich and Hartmann 1938). Morgan's analyses are of the water soluble form.

red corpuscles

Schiøne R. (1944). Act. pathol. microbiol. scand 21, 401-410.

Freudenreich V. and Hartmann C. (1938) Zts. f. Immunitätsforsch. 92, 141

Pauling L., Itano H.A., Singer S.J. and Wells I.C. Science 110, 543.

Perutz M.F. and Mathieson J.M. (1950) Nature 166, 677.

with about 1% of encephalin

other baptisms. Other people secrete the H substance, and also the A and B substances if they make them. ~~This is appears also to coat other substances off certain cell membranes, or to render membranes impermeable to them. Although it may be regarded as making them permeable.~~ Another surprising of an antigenic gene is
~~effect on permeability cell membranes~~, but A_2 cells are less readily hydrolysed by hydrolase substance than A_1, O, or B. (Schone 1944)

There is similar evidence that different types of haemoglobin are made by different alleloworphic genes. A gene si si, which is not rare either in African or among American negroes, produces (in America, but not certainly in Africa) a fairly fatal anaemia when homozygous. The haemoglobin in these corpuscles of patients forms solid crystals on reduction, and distorts the corpuscles into shapes which have been compared to sickles. The si si genotype is called sickle-cell anaemia. The heterozygotes Si si are fairly normal, but may sometimes have a slight anaemia. However if their corpuscles are coated haemoglobin is completely reduced, some of the corpuscles are distorted, and the "sickle cell test", which is the name given to the heterozygous genotype, can be detected. It is very common throughout Africa south of the Sahara. Pauling, Itano, Singer, and Wells (1949) made the very remarkable discovery that the carboxyhaemoglobins and the reduced haemoglobins of normals and sickle cell anaemics differ in their mobilities in an electric field, the latter being more basic, the isoelectric points differing by about .23 of a pH unit. Perutz and Mitchison (1950) found that the two oxyhaemoglobins have about the same solubility, and but reduced sickle cell haemoglobin is ~~about equal~~ much less soluble than normal

Table. 2

Type of haemoglobin	Normal	Sickle	Rare	Rare	Foetal	
	a	b	c	d	f	
Solubility or reduced solubility	+	−	+	+	+	
Mobility at pH 6.6	+	++	+++	++	+	
Resistance to alkali	−	−	−	−	+	
Genotype						Phenotype
S_aS_a	100	0	0	0	0	Normal
S_aS_b	55-44	23-45	0	0	0	Sickle-cell trait
S_bS_b	0	95-100	0	0	0-25	Severe anaemia
S_aS_c	+	0	+	0	0	? Normal
S_bS_c	0	+	+	0	±	Moderate anaemia
S_aS_d	+	0	0	+	0	? Normal
S_bS_d	0	+	0	+	±	Moderate anaemia

F 27

Table

Genotype.	Types of haemoglobin %			
	Normal.	Sickle	Rare types	Foetal
$S S$	100	0	0	0
S_S	55-99	23-45	0	0
ss	0	95-100	0	0-25
$S s^{1,2}$	+	0	+	0
$s s^{1,2}$	-	+	+	+

or two or three less glutamine or aspartic acid.

Schroeder, W. A., Webb Kay, L. M., and Wells I. C. (1950) JBC 184, 221

Perutz, M. F., Lepori A. M. and Ennis F. (1951). Nature 167, 924.

Ikano H. H, and Neel J. V. (1950). PNAS 36, 613

Ikano H. A. (1951). PNAS. 37, 775.

haemoglobin. ~~It might~~ The haem is certainly the same, so the difference must be in the globins. Everything would be explained if each molecule of sickle cell haemoglobin contained two or three more lysine or arginine residues. The analytical data of Schroeder, Kay, and Wells (1950) show that they do not differ in this way, though they may contain more serine and threonine, less leucine and valine. More remarkably Perutz, Liquori and Eirich (1950) were quite unable to distinguish the diffraction patterns of the two haemoglobins.

So far as I know, nobody has made the experiments which would have been obvious thirty years ago, namely to compare the oxygen and carbon monoxide dissociation curves of these haemoglobins and to measure their spectra as accurately as possible.

~~The matter was~~ The matter was rapidly complicated by a series of further discoveries by Itano and Neel (1950) and Itano (1951). Families were discovered in which a child had not very severe sickle cell anaemia, and only one parent had the sickle-cell trait. The other parent was found to have two electrolytically separable types of haemoglobin. One agrees with sickle cell haemoglobin in its electrophoretic behaviour, but not its solubility, the other is even more electronegative, but soluble when reduced. It appears that these are due to other alleles of the same locus as the gene for sickle cell anaemia. Finally anaemics may have up to 25% of foetal haemoglobin. This forms about 80% or more of the haemoglobin in normal babies at birth, and usually disappears at about 4 months. It can be distinguished by its high resistance to alkali denaturation, but has ~~the same~~ solubility much the same solubility and

appears to be a mixture of normal ~~adult~~ (a) and foetal (b) haemoglobin, according to Rush (1951). Thus the gene produces a chemical change, apparently by reducing the production of normal haemoglobin, the foetal being produced in adult life as a physiological compensatory factor.

Rush A. (1951) P.N.A.S. 37, 187

Douglas C. D., Haldane, J. S., and Haldane J. B. S. (1912) Journ Physiol 44, 275.

electrophoretic mobility as normal haemoglobin. It is also found in several other types of anaemia, and its continued production may be regarded as an adaptive response. Table 2 summarizes the situation. Haemoglobins a, b, c, and d appear to be produced by allelomorphic genes. It is not yet known why the mixture of b haemoglobin with c or d is ~~relatively~~ less soluble than the mixture with a. ~~The~~ We are not know whether c c and d d homozygotes are normal. The haemoglobin in some other congenital anaemias, for example thalassaemia (Cooley's anaemia) ~~has apparently not been investigated~~

All these facts could be explained on the hypothesis that each of the four genes concerned manufactures its own type of globin independently of the others, except that there is a certain amount of competition, the normal allelomorph usually making rather more of the total than the sickle-cell haemoglobin-making gene. This hypothesis, which does not accord with the findings of Callan (cf Chap 8) on the permeability of nuclear membranes, has the merit of being disprovable. ~~If abnormal haemoglobins are found in other combinations, then either they must be alleles of the~~ ~~two genes, or several genes at several loci are concerned in haemoglobin synthesis.~~ There is however a simpler way of solving the question. Douglas, Haldane, and Haldane (1912) found that when the haemoglobin of an individual was fully saturated with CO and O_2 the ratio of the affinities for these two gases was constant, and independent of pH, dilution and so on. The ratio remained constant for a given individual over some years. For J.S. Haldane it was 300:1, for C.G. Douglas 246:1, for mice A, B, C, and D 164:1, 139:1, 222:1, and 150:1

A Also occurs in rabbits and horses, as well as between species, and

Barcroft J. (1914) The Respiratory function of the Blood. Vol 2. Cambridge

Haldane J.S. and Priestley J.G. (1935) Respiration Oxford

Fox, H.M. (1945) Nature 156, 12.

B Fox found spectroscopic differences between the haemoglobins of different rabbits, but not between those of different frogs or earthworms, nor, conversely wrongly, of different men.

21a (in Vol. 2)

Filitti-Wurmser, S., Jacquot-Armand, Y., and Wurmser, R. (1950). Journ. Chim. Phys. 47, 419.

Bancroft (1928) and his colleagues showed that these differences are highly correlated with differences between the positions of absorption bands in the spectra of oxyhaemoglobin and carboxyhaemoglobin. Haldane and Priestley (1935) went so far as to state that "It does, in fact, appear to be fairly certain that each individual has a specific kind of haemoglobin just as he has a specific nose", and to cite Todd's results as an analogy. This may well be an exaggeration, but there are certainly many types of haemoglobin within a species. It is possible that they are all determined by genes at the same locus as that for the sickle-cell character and the Hörlein and Weber's methaemoglobinaemia. If this is proved to be so it will be highly probable that haemoglobin is a primary product of gene at this locus. If, as I think more likely, several different loci are concerned, we shall either have to suppose that the haemoglobin molecule is made up of parts manufactured by several different genes, or that it is passed from one gene to another like a car along a conveyor belt, or adopt some other such hypothesis of cooperation.

Even if the hypothesis of the independent production of different globins by different genes is confirmed at this level, it has been disproved at another level. Erlitz-Wiener, Sargent-Armand, and Wiener (1950) have done a large amount of quantitative work on the B, or anti-B, agglutinin of human group A bloods, and its combination with the B agglutinogen. To obtain reproducible results it is necessary first, by heating the serum to 56 °C for 30 minutes, to destroy a thermolabile component of complement which partially inhibits agglutination. When this has been done, provided that a number of further precautions are taken, the results are extremely

(go on to next volume)

Hörlein H. and Weber (G.) (1948). Deutsch. Med Wschr. 73, 476.

The dominant inheritance of this character suggests the possibility

Another type of pathological globin is known. This occurs in a family described by Hörlein and Weber (1948) in which methaemoglobin-reductase was absent or dominant. About 20% of the haemoglobin was present as methaemoglobin, whose absorption spectrum differed from that of normal methaemoglobin. By exchanging its haem with that of normal haemoglobin the difference was shown to lie in the globin. The condition did not respond to methylene blue like the Irish type of methaemoglobinaemia discussed in chap. 6. Unfortunately it is not yet known whether a mixture of two haemoglobins as is present. It is probable that other cases of this genetically determined haematological abnormality will be found to have abnormal haemoglobins. If so then intermarriage with persons with the variable cell count will furnish information as to the number of loci involved.

The use of Neurospora for biochemical purposes is largely due to Beadle, who has also summarized the ~~results~~ earlier results (Beadle 1945). ~~The~~ An Admirable account both of the genetics and much of the later biochemical work is given by Catcheside (1944) and Horowitz (1950) ~~whose book is cordially recommended both to biochemists and geneticists~~. Aspergillus nidulans will grow on a mineral medium containing no organic substance but glucose, and with nitrate, sulphate, and phosphate as N, S, and P sources. (Pontecorvo 1952)

Beadle G. W. (1945). Chem Rev. 37, 15

Horowitz N. H. (1950) Advances in genetics 3, 33.

Chapter 3.

Genes controlling ~~nuta~~ synthesis in fungi

All normal strains of

Neurospora crassa ~~all normals~~ grow on a "minimal medium" consisting of water, inorganic salts including sulphate and nitrate, glucose and a trace of biotin. It will grow somewhat better on ~~a med~~ the minimal medium ~~con~~ plus yeast extract, malt, and autolysed Neurospora mycelium; this is called the "complete medium". A large number of mutants will grow on the complete medium, but slowly or not at all on the minimal medium. These auxotrophic mutants may be called conditional lethals. Probably ~~most of~~ many of the metazoan lethals are conditional. Thus a lethal ~~anaemia in mice~~ anaemic form of mouse can be kept alive by transfusion, a sterile dwarf form can be made to grow and the males rendered fertile by injecting anterior pituitary hormone, and so on. ~~In the case of the mould mutants etc.~~

Mutations can be induced by treating the macroconidia (uninucleate asexual reproductive cells) with X rays, ultraviolet radiation, or chemical mutagens. These may produce upto 50% mutants, but only in doses which kill the majority of the spores. Several methods are available for picking out mutants in various fungi and bacteria.

1. Isolated spores are grown on complete medium, and a subculture from each such culture transferred to minimal medium. Those whose subcultures fail to grow are kept. This is a laborious method.

2. Spores are sown on a minimal medium, and those which don't grow are picked out before they die.

3. Spores are grown on minimal medium and filtered after a few hours or days. The filtr

Davis B. D. (1948). I~~solation of biochemically deficient~~ mutants of bacteria by ~~penicillin~~. J Am. Chem. Soc. 70, 4267

~~a~~ mostly requiring amino-acids

Fries

Bonner D. (19)

In fact of course a completely vitamin free protein digest is not readily obtained.

and Penicillium-~~clusters~~ (Bonner 19)

~~The~~ Bonner's technique did not enable him to distinguish between inability to ~~d~~ reduce sulphate and to synthesize cysteine.

strains those which have grown before.

4. Some ~~mould and~~ bacteria (not Neurospora) are killed by penicillin only when growing. Minimal medium plus penicillin selects the mutants, which can then grow on complete medium without penicillin (Davis 1948)

5. ~~In~~ Aspergillus spores needing biotin generally die in a biotin-free medium in 4 days. Those needing biotin and thiazole ~~live longer~~ so or some other substance live longer. Thus of 4×10^6 untreated biotin-needing spores 5·2 survived for 14 ½ hours. Of these 22 were further mutants (Pontecorvo ~~and Macdonald+q~~ et al. 1952), Thus is an observation of some evolutionary importance. Once a species has started on the path towards parasitism by losing some synthetic capacity it may survive longer in an unfavourable medium if it has lost still other capacities, and is not, so to say, tempted to grow.

Having obtained a stock which grows on the complete but not the minimal medium two more steps must be taken. The order is irrelevant. It must be established that the mutation is due to a single gene. This is done by crossing it to a normal stock and showing that just half the spores in each ascus resemble either parent. And the mutant step at which needs must be established. This ~~again~~ can be done in several steps. We can add a vitamin-free protein digest or a yeast extract to the mineral medium and see whether either of these will sustain growth. Table 4 shows the results of this type of analysis for two fungi, Ophiostoma multitransmutalum (after Frais) and Aspergillus nidulans (after Pontecorvo 1952). The former was grown in a mineral medium containing NH_4^+, the latter in ~~a~~ two containing NO_3^-).

The most surprising feature is perhaps that almost over half the

A In fact 28 grew on sulphite, and the other 16 on thiosulphate.

B Pontecorvo has also shown that the details of the process of selection make a great difference to the proportion of auxotrophic mutants of different types which are found. Nevertheless real differences occur. Pontecorvo has failed to obtain inositol requiring mutants in Aspergillus, though they are common in some other fungi.

C Still another type of auxotrophic mutant is known, which will grow neither on the minimal nor the complete medium, but will grow on the minimal medium plus a supplement. For example Pontecorvo was unable to obtain a "tryptophanless" mutant by his standard methods. He obtained one by growing on minimal medium plus tryptophan, but its growth is inhibited by other amino acids.

It is important that almost all mutants require a single supplement. Out of 612 tested by Pontecorvo, only 33 which would grow on complete but not minimal medium have failed to respond to a single substance. They include auxotrophous mutants (e.g. thiamineless and lysineless) and mutants requiring supplements not available (e.g. perhaps vitamin B(12). The small number undescribable is a testimony to the completeness of our knowledge of elementary biochemistry.

Table 4

Need	Ophiostoma	Aspergillus	Penicillium
Amino-acids	178	~~34~~ 40	250
Nucleic acid components	61	~~19~~ 145	19
Vitamins	151	~~18~~ 34	55
Reduced S	74	~~64~~ ~~209~~ 305	→ ?
Reduced N	→ ?	~~9~~ 20	31
Total	464	~~134~~ 547	355

mutants in Aspergillus all grow in presence of sulphide, cysteine, etc, but not of sulphate.[A] This however does not imply that there are a great number of processes concerned in sulphate reduction. It is more probable that there is one which is particularly sensitive.[B]

The next step, if possible, is to make the nature of the requirement more precise, for example to show that the mutant will grow in minimal medium plus arginine or ~~nicotinic~~ riboflavin. Beadle's school denote a mutant requiring arginine as "arginine-less". This terminology has the merit of brevity, but it is unfortunate for two reasons. In the first place it suggests that these mutants lack arginine or that they cannot synthesize it. Secondly it is ambiguous. If an "arginineless" mutant will grow on ornithine it might as well, or better, be called "ornithineless".[C]

Having roughly classified ~~one~~ the mutants, the next step is to see whether the same ~~process is a loss is concerned in~~ normal gene is altered in two similar mutants. In Neurospora we can cross them. If all the progeny of two

In other species crossing is more difficult, or even impossible. Pontecorvo has so far only analysed about 60 of ~~his~~ over 600 mutants genetically, and has only localised 27 on chromosomes. This is however no mean achievement. It is about the number localised in domestic poultry in half a century. But it means that, for example of the ~~44~~ five "ornithineless-arginineless" auxotrophs which he obtained ~~of table~~, one can only say that at least two are at different loci.

Srb. A, and Horowitz N. H. (1944) J. Biol. Chem 154

Bonner D (1946). Am. J. Bot 33, 788.

Fry in Vol 2

"arginineless" mutants are arginineless, it is generally assumed that the same gene is affected in both. This is however not necessarily so. Two genes concerned in different steps of the same synthesis are often close together on a chromosome. This means that ~~there is no~~ recombination, which would give a "prototroph", that is to say an organism which will grow on the minimal medium, occurs very rarely. ~~Another~~ Two other methods of ~~obtaining~~ getting similar information are as follows. We can grow the two in a mixed culture. If one of them liberates a substance which the other can use but not make, they may be able to live together. In some species, especially the unusual ones, hyphae may fuse to give a "heterokaryon", an organism containing two different kinds of nuclei. This may be capable of life on the minimal medium. Heterokaryons are far from being mere laboratory artefacts. All the strains of Penicillium notatum used for making penicillin are heterokaryons.

~~Later~~ Further steps are the location of genes at definite loci on different chromosomes, and the ~~final~~ analysis by various methods of their biochemical actions. A classical case is the analysis by Srb and Horowitz (1944) of 15 arginineless mutants of N. crassa. Eight were considered to be duplicates. The other 7 gave the results shown in Table 5, along with similar results obtained by Bonner (1946) in Penicillium notatum and by Pontecorvo (1952) in Aspergillus nidulans. It is clear that in the first five species there is full agreement with the hypothesis (Fig. 1) that arginine is synthesized from ornithine through citrulline, as it is in some (but perhaps not all) mammalian livers. In arg-1 the step from citrulline to arginine is blocked, in arg-2 and arg-3 that from ornithine to citrulline. Therefore

Table 5

Species	Mutant	glutamic acid	proline	ornithine	citrulline	arginine
Neurospora crassa	arg 8, 9	?	+	+	+	+
	arg 4, 5, 6, 7	?	−	+	+	+
	arg 2, 3	?	−	−	+	+
	arg 1	?	−	−	−	+
Penicillium	24033	+	+	+	+	+
	35784	−	+	−	−	−
notatum	9924	−	+	+	+	+
	6155	−	−	+	+	+
	6542	−	−	±	+	+
	3485	−	−	−	−	+
Aspergillus	one	+	+	+	−	+
	fourteen	−	+	+	−	+
nidulans	five	−	−	+	−	+
	one	−	−	−	−	+
	one	−	−	+	−	−
	two	−	+	−	−	−

If it is so we must assume that the mycelial membrane, though permeable to ornithine and arginine, is impermeable to citrulline, which seems very improbable. Moreover markedly the mutant "ornithineless" and the true "prolineless" mutants cease to show that neither ornithine nor proline are intermediates ~~in anything~~ in arginine synthesis, even though most of the "arginineless" mutants can utilize either of these substances

(in Vol 2)

be two steps, or perhaps two genes are needed to make an enzyme, or one to make an enzyme and another a coenzyme. On the other hand citrulline is apparently not an intermediate in A. nidulans. ~~The most plausible hypothesis~~ ~~There is perhaps that arginine is made from ornithine and urea by arginase acting in reverse. But on thermodynamical grounds we would be inclined to postulate an energy-rich substrate such as phospho-urea. It is equally clear that this process does not occur in the other two species on a scale sufficient to support life~~ growth.

The table does not prove that citrulline is an intermediate in the first two species. For if so it would prove that proline was one. The mutant 35784 in Penicillium requires proline, and we must suppose that the function lost is the capacity for reversible transformation of proline into an intermediate. In 6542 the synthesis of citrulline from ornithine appears to be difficult.

Table 6 (after Catcheside ~~and others~~ ~~Emerson, Horowitz and others~~ and others) records all the biochemical mutants of Neurospora crassa which had been located on the chromosomes up to about 1948. It reflects to some extent the interests of the workers concerned, and does not include, for example mutants requiring acetate, nitrite, and sulphanilamide. The list is however clearly far from complete. It only includes a few of those which, although requiring some constituents of the complete medium, are inhibited by others. ~~It includes no mutant~~ No attempt has been made to find mutants which require such substances as the glucose phosphate, which have disappeared from the complete medium as the result of autolysis, still less those requiring substances to which the hyphal membrane is impermeable. Probably most mutations do not completely suppress growth on minimal medium.

Fincham. J. R. S. (1950). JBC 182, 61

Fincham worked with a mutant 32213 (= 47305) which will grow on any of 15 amino-acids, none of the tested though but on no keto-acids or D-amino-acids. Growth is particularly good with glutamic and aspartic acids, and ornithine, but does not occur with glycine, serine, threonine, lysine and some others. Ammonia tends to accumulate in the medium. This mutant, like the wild type, has at least two transaminases. It apparently lacks some part of the glutamic dehydrogenase system, with which a normal mycelium makes glutaconic acid from ammonium and 2-ketoglutaric acid.

Mitchell H. K. and Houlahan M. B. (1948) J. Biol. Chem 174, 883

↑ probably by reducing the amide

↑ nor will any of the lysineless Aspergillus mutants.

Wender E. (1951). J. B. C. 192, 609

Particular cases of

Before we consider amino-acid synthesis let us begin with a elementary point due to Fischer (1905). A mutant ~~strain~~ shown to "need" a given amino-acid will generally grow on the corresponding α-keto-acid. This has been shown for methionine, valine, leucine, and arginine. Where an α-keto-acid is not available such a mutant will often grow on the corresponding D-amino-acid, since Neurospora possesses powerful D-amino-acid oxidase. This however will not attack D-serine, D-lysine, or D-tryptophan, which are therefore useless to ~~mycelium~~. Another mutant has appeared which cannot use ammonia to form L-amino-acids, except after training (see Chap.). This mutant will grow on any of 15 L-amino-acids, particularly well on glutamic and aspartic acids, presumably using a transaminase, of which Neurospora has at least two. If supplied with D-amino-acids, it merely poisons itself with ammonia which it cannot use.

Lysine is ~~however~~ a special case (Mitchell and Houlahan 1948). Mutant 33393 (lysineless) will grow on lysine, or on L- or DL-α-amino-adipic acid. That is to say it can attack an amino group to the ε carbon atom, ~~with elimination of a water molecule~~. ~~Three other lysineless mutants will not grow on α-amino-adipic acid. This suggests that the~~ But it will not grow on α-keto-adipic acid. (4544, 15069, and 37101) Three other lysineless mutants will not grow on α-amino-adipic acid, and ~~the block~~ it is possible that three stages in its conversion to lysine are blocked in them. It is surprising that D-α-amino-adipic acid seems to be fully utilized in presence of its L-isomer, while the keto-acid is not used. Windsor (1951) later showed by N¹⁵ labelling that α-amino adipic acid is ~~the~~ converted into lysine, and no other amino-acid, by 33933.

Teas H.J., Horowitz N.H., and Flury, M. (1948). J.B.C. 172, 51

Horowitz N.H. (1947) JBC 171, 255

*[Footnote] J. By L-threonine I mean the protein constituent L_s-threonine, related to L-serine or D-threose, not L_g-threonine, related to L-threose or L-glyceraldehyde.

Teas H.J., J.B.C. 140, 389

[It may be remarked that cystine can often (? always) replace cysteine, but homocystine will not replace homocysteine, though DL-homocystine thiolactone will do so.

^ each of which has one "working-glass" enol or alcoh.

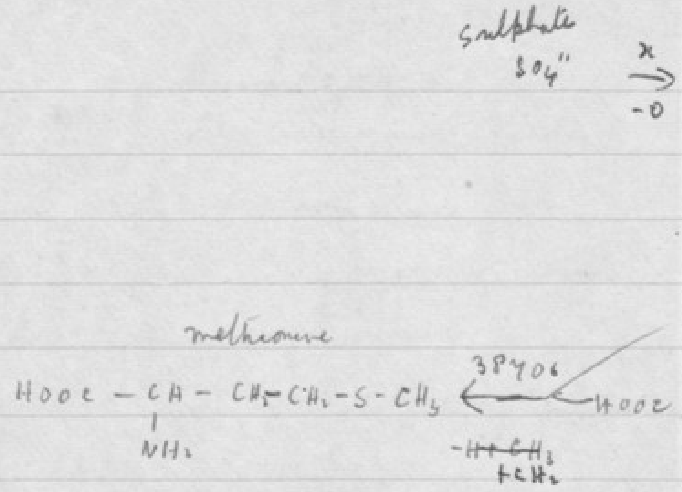
In the rat's liver homocysteine and serine give cystathionine and thence cysteine and homoserine, that is to say two metabolic steps occur in the reverse direction.

Let us now consider in further detail the synthesis of some amino-acids. A group of at least 12 different mutants ~~require some~~ will not grow on minimal medium, but require some or all of cysteine, methionine, and threonine. [The unexpected appearance of threonine* in this context was due to the discovery by Tatum, Horowitz and Fling (1948) that mutant 51504 needs D- or L- or D- methionine, and L- threonine. They later found that it could grow on L- homoserine, or on L- threonine plus D- homoserine or B- hydroxy- L- homoserine. Fig 1 shows the various relations revealed by their work and that of ~~Horowitz (1947)~~ others

Most unfortunately the "homoserineless" mutant 46003-R will grow on canavanine (Tatum 47)

*The number above an arrow is the number of the mutant in which the corresponding step is blocked. The symbols below it represent the chemical change, a reduction, oxidation, hydrolysis or its reverse, or a methylation.] The path from cysteine + homoserine to methionine was studied by Horowitz (1947). H 98, when grown in presence of 25 mg / litre DL-methionine, accumulated a substance, namely the mycelium which supported growth of 36104. The yield was 360 mg. per kilogram wet weight, and it was shown to be L- cystathionine, which has 3 optical isomers. Both D and L- allocystathionine had a slight effect in supporting the growth of 39816, in which the block occurs before the cysteine stage: D- cystathionine, which has two 'unnatural' carbon atoms, was quite ineffective: It will be seen that this rather elaborate process is used to transfer a sulphur atom from the three- carbon chain of cysteine to the four- carbon chain of homoserine. [~~It is naturally but 39706 seems to be specific for the methylation of homocysteine. Later several allelomorphs methylated any other~~

From top 8a



[Presumably the step blocked in H98 produces serine as well as homocysteine. But we cannot hope to find a mutant in which serine accumulates. For a block to the utilization of any serine would prevent normal protein synthesis, and a gene causing it would be an unconditional lethal.

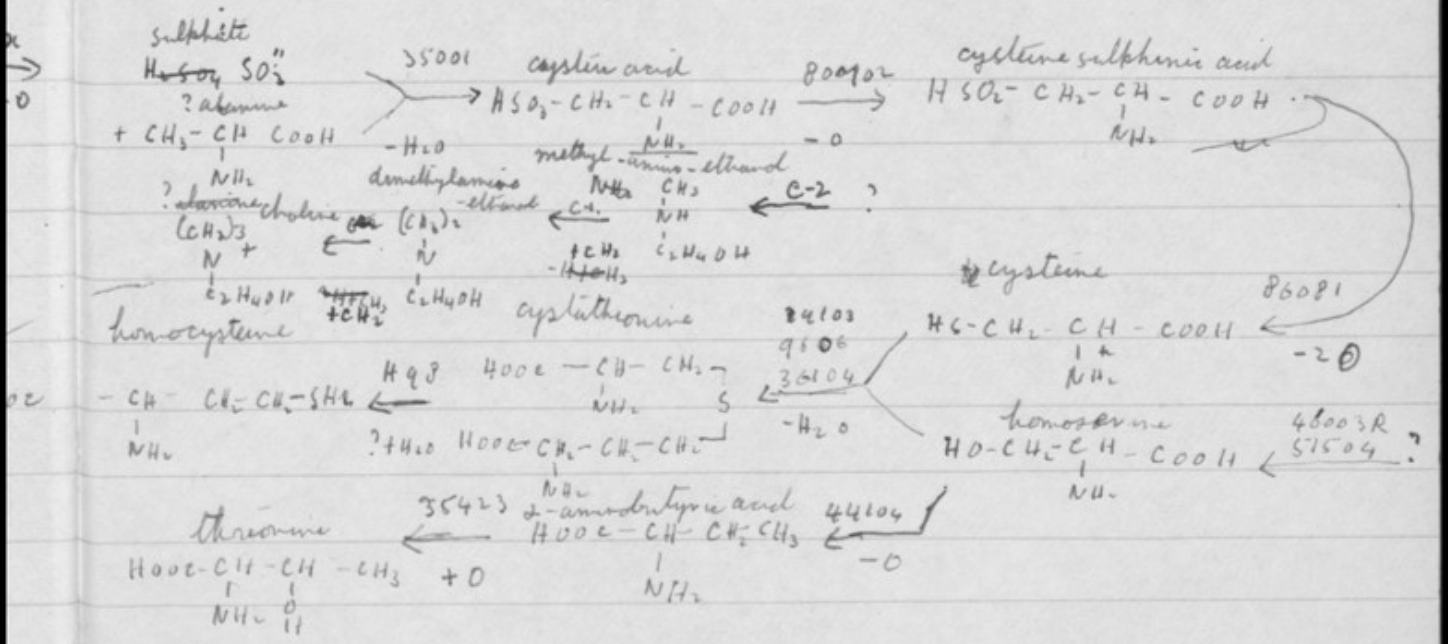


Fig 1

—— H. Linnane, Horowitz (45-0)

~~compounds, 38 [illegible] as methionine. The origin of the methyl groups is unknown.~~ It is impossible to describe all the experimental work to substantiate in Fig. 1 It is however notable that L- homoserine has been isolated from cultures of 9606 supplemented with threonine and methionine.

A ~~very~~ especially remarkable series of researches is summarized in Fig. 2, though it must be emphasized that it is far from complete. The first observation made was that some mutants could be kept alive either by a protein digest or B. vitamins. The seed was Further precision showed a need for tryptophan or nicotinic acid, The nicotinamide being equally useful. Other mutants require ~~only~~ the nicotinic acid but can make tryptophan. Anthranilic acid was detected by its fluorescence and later isolated from a "tryptophanless", 10575. The other intermediates were similarly

Mitchell H. K. and Nye J. F (1948) PNAS. 34, 1

Bonner D. (1948) PNAS. 34, 5.

Bonner, D. and Yanofsky C. (1949) PNAS 35, 5-476

Haskins and Mitchell (19)

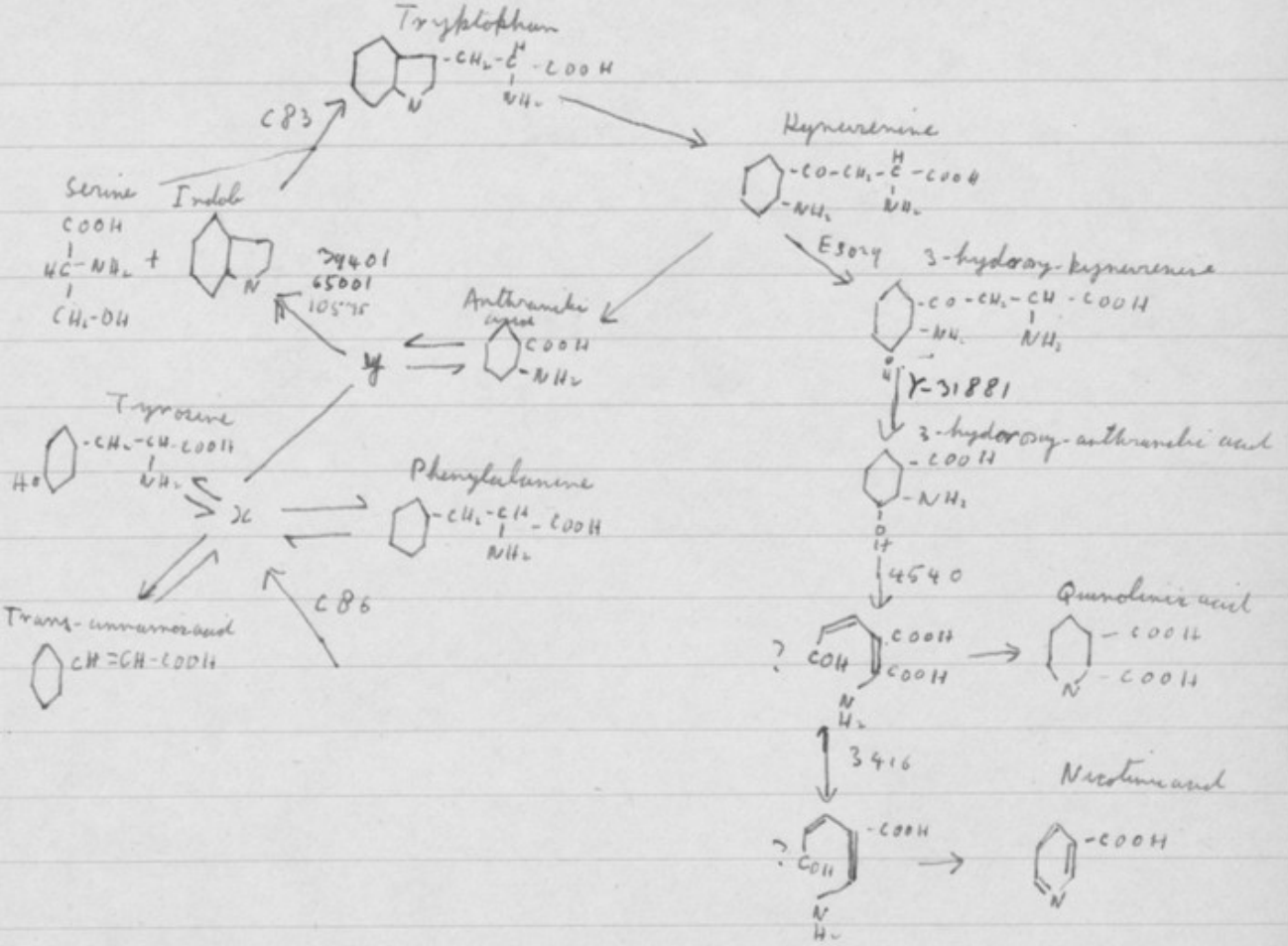


Fig 2

Based on ~~Bonner (1948)~~ Mitchell and Nye (1948), Bonner (1949), Bonner and Yanovsky (1949), Hankins and Mitchell (19), etc. The aldehydes intermediate between 3-hydroxy anthranilic and nicotinic acids are hypothetical

(Butenandt, Wendel, Wenchert, and Dorjugin (1943)

Butenandt, A, Wendel W., Wenchert R, and Dorjugin W (1943). Zeit physiol Chem 279, 29.

Bonner D. M., Yanofsky C., and Partridge C W. H (1950). PNAS 38, 25.

detected and shown to replace tryptophan or nicotinic acid. 3-hydroxy-kynurenine had been isolated by [illegible] from mutant Drosophila (see Chap)

3416 produces quinolinic acid from tryptophan, and this is clearly not an intermediate, as none of the nicotinamide requiring mutants can use it. Anthranilic acid is accumulated, though not in large amounts, by some mutants when supplied with kynurenine, but the details of the cycle are far from clear. Isotope studies show that in mutants where tryptophan synthesis is blocked, all the N atoms of nicotinic acid are derived from the ring N atom of tryptophan

Nevertheless Bonner, Yanofsky and Partridge (1952) have shown that the notion of metabolic blocks, while heuristically valuable, is much too simple. 3401 will grow on nicotinic acid alone, and of course nicotinic acid, can then make tryptophan from labelled anthranilic acid. Similar experiments were made with other mutants, control with 3416, which diverts the precursor of nicotinic acid into quinolinic acid. They were given unlabelled nicotinic acid and N^{15} labelled tryptophan, indole, or anthranilic acid. C-83, 3416 showed a complete block. When fed on N^{15} labelled tryptophan, both the tryptophan and quinolinic acid isolated from it has 94-99% of the expected N^{15} content. But 45001, 3416 and 7655, 3416 behaved differently. Both can normally make tryptophan from indole and anthranilic acid, but not from mineral medium. Under various conditions $\frac{16}{32}$-80% of the tryptophan and 29-100% of the quinolinic acid was derived from the labelled source. Various possibilities such as reversion and N exchange were ruled out

It was concluded that a number of mutants, though they cannot

Emerson S. (1950) Cold Spring Harbor Symp. quant. biol. 14, 40.

and Kinsford

The source of the methyl groups in choline, which arises ~~from~~ be produced by further methylation of amino-ethanol, is unknown.

Many more additions could be made to Fig 1, mainly as the result of work by various authors summarized by Emerson (1950). In three different mutants the step from homoserine and cystathionine is blocked. Each of them accumulates a substance or substances (presumably other than cysteine and homoserine) which will allow 51504 to grow without methionine or threonine, and which may be homoserine. The cystathionine precursor may be optically inactive at least as regards the α carbon of homoserine, since it is formed from D-homoserine. Further 9666 accumulates threonine. The methylation of homocysteine is a complex process blocked in nodes than ten mutants, whose allelomorphism is under investigation. The methyl probably comes from an choline, since C_1 and C_{1-2} can grow for a time, but not indefinitely, on methionine alone, and 32603 will grow well on choline or methionine, but not on homocysteine. The evidence for the complexity of the methylation process is as follows. Most mutants, e.g. 86081, 9666 and H93 can reduce selenate. One of the mutants blocking methylation always reduces selenate. Five others can only do so if extra methionine is added. Three more can never do so. Finally p-aminobenzoic acid is somehow involved in methylation, since methionine, but not homocysteine, reduces the p-amino-benzoic acid requirements of the p-aminobenzoicless mutants.

1833. To return to this point later (p.)

Wagner R.R. (1944) / PNAS 35, 185

~~Pontecorvo, G. (1950) Biochem. J. 46, 40~~

or (unlike N. crassa for a mutants) an unidentified acid

3-hydroxy-kynurenine

grow on mineral medium without a substance, or yet make this substance when growing. The authors describe this phenomenon as "leakage".

This phenomenon must be considered in connexion with Wagner's (1942) finding that ~~mutants~~ although certain mutants need pantothenic acid, and cannot synthesize it from pantoyl lactone and β-alanine, they ~~the~~ an enzyme system which carries out this synthesis can nevertheless be extracted from them. This decisively disproves the one-gene-one-enzyme hypothesis as being universally valid. There are many possible reasons for blockage in a mutant, one being the presence of an inhibitor.

Once again, other organisms have different synthetic paths. Pontecorvo (1952) obtained ~~three~~ nine mutants in Aspergillus nidulans which will grow on mesbine acid. ~~Two are leaky~~ Four of these will also grow on anthranilic acid, indole, tryptophan, kynurenine, or 3-hydroxy-anthranilic acid, though one requires very large amounts of tryptophan or anthranilic acid. ~~One~~ The other four, however, which will grow on 3-hydroxy-anthranilic acid, or on very large amounts of anthranilic, will not grow at all on tryptophan or kynurenine. ~~This is doubtful whether it will grow on indole.~~ It is indole; This suggests the presence of a not very efficient "tryptophan-bye-pass", allowing the direct oxidation of anthranilic acid, in place of the very round-about route of Fig 2.

Bonner D. (1946). J. Biol Chem. 166, 4545.

Adelberg E. A, Bonner D. M., and Tatum, E. L. (1951). J. B. C. 190, 839

Tatum E. L. and Adelberg E. A. (1951). J B C 190, 843

Wright B. (1951). Amer Biochem 31, 332.

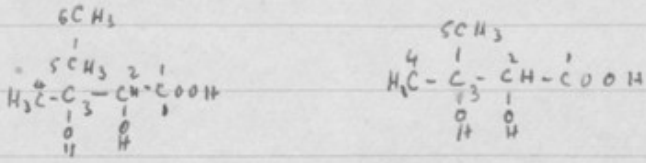
Umbarger H. E., and Adelberg E. A. (1951). J. B C. 192, 883.

Umbarger and Adelberg (1951) have made it highly probable that these acids are converted to the corresponding keto acids by the normal enzymes. This interesting molecular rearrangement is blocked in 1611 y. Perhaps at larks an isomerase

Wright (1951) found that a mutant requiring serine or glycine grew satisfactorily on glycollic or glyoxylic acid.

Mitchell M. B. and Houlahan M. B. (1946) Fed. Proc. 5. 370.

Similar investigations into the origin of valine and isoleucine (Bonner 1946, Adelberg, Bonner, and Tatum 1951, Tatum and Adelberg 1951) yielded the following results. 16117 needs valine and isoleucine for growth. 33051 grows at about half the normal rate on valine, while isoleucine brings the rate up to normal. In each case growth is best when the two acids are present in about equimolar concentrations, and it became clear that the requirement of valine by 16117 was due to the accumulation of an isoleucine precursor. This turned out to be 2-β-dihydroxy-β-ethylbutyric acid. The corresponding methyl compound is a valine precursor. When the carbon source was C-labelled acetate it was found that carbons 5 is mainly derived from the methyl group of the



Isoleucine precursor Valine precursor

Fig. 3

acetate, which also contributes to carbons 1 or 2 and 4, but not to 3. Similarly the carboxyl group contributes to 6, to 1 or 2, and to some extent to 3, but not to 3 or 5.

I must pass over a large amount of work on purine and pyrimidine synthesis, merely noting a few facts. Adenine-requiring mutants in this and other species often accumulate pigment, and are thus identified without special methods. Thus adenineless 35203 accumulates a purple pigment, which is probably formly close to adenine, as three other adenineless mutants, when combined with 35203, suppress its formation. (Mitchell and Houlahan 1946). Two points come out clearly from the

Lowry, H.S. and Pierce J.G (1944). J.B.C. 153, 61.
Mitchell, H.K., Houlahan M.B. and Nye J. F. (1948) JBC 172, 525.

[A] However Mitchell Michelson, Dodd and Mitchell (1951) isolated orotidine, orotic acid riboside, from 3600 l (hypoxanthine) in a yield on newly 1% of its dry weight

Michelson A.M., Dodd W. and Mitchell H.K. (1951) P.N.A.S 37, 391

[B] Mitchell and Houlahan (1947) find that oxaloacetic acid and aminofumaric diamide will support a rather slow growth in some of these mutants.

Mitchell H.K. and Houlahan M.B. (1947) Fed. Proc. 6, 506.

work on pyrimidineless mutants. Orotic acid (4-carboxy-uracil) can be a substitute for uracil in some, (Lowry & Pierce 1944) and accumulate in another (Mitchell Houlahan and Nyc 1948), reaching a concentration of 1·3 gm/litre, which is almost saturated, in the culture medium. It is not necessarily a normal intermediate. As a result of this work it has been shown to play a part in mammalian pyrimidine metabolism. Secondly Houlahan and Mitchell (1951) find that several lysineless mutants accumulate pyrimidines. The significance of this is not clear.

I must now refer to a characteristic which is very common in these biochemical mutants. This is the competitive inhibition of their utilization of the needed substance by related compounds which does not inhibit the growth of normal mycelia. Thus Pontecorvo (1951) studied 8 (not necessarily distinct) all mutants of Aspergillus nidulans all "arginineless", i.e. with growth restored by arginine. All of them were inhibited by lysine at a concentration about twice that of the arginine. Lysine also inhibits their growth on or nitrate where this is possible, but where growth on proline is possible, lysine actually stimulates it. ~~Such facts as these are, I think, only intelligible if one~~ This seems to imply that arginine or a product of it competes with lysine or a product of lysine ~~competes with arginine supplied from~~ That is to say lysine competes with arginine or a nitrate supplied from outside, but not with arginine formed by the normal synthetic process. Probably the uptake of amino-acids by a hyphae is a process as complicated as their reabsorption by the human kidney tubule (see Chap.) and will have to be investigated on similar lines. Competition may of course also occur for intracellular enzymes. But it is clear that

Emerson S 1948. J. Bact. 54, 145

Zalokar M. 1948. PNAS 34, 32

~~Emerson S. 1950.~~

Hoagland MB, and Mitchell H.K. (1948) Arch. Biochem 14. 259.

the genetic blocking of a reaction may very well be due to the accumulation of an intermediate

A very remarkable case was investigated by Emerson and Zbarsky (1944) Several sulphanilamide resistant forms were obtained by selection. One was found (sfo) was found to need sulphanilamide (about 10^{-4} M) at high temperatures. The normal mould is inhibited in minimal medium + .01M sulphanilamide, and grows slowly if even 10^{-6} M p-aminobenzoic acid is added. Another mutant (pab) needs p-amino benzoic acid.

On combining the two we get the situation shown in Fig 3. where the time needed to grow 1.5 mm. is plotted against concentration of p.-amino-benzoic acid. sfo is poisoned by too much of this substance. Later Emerson (1950) discovered that methionine and threonine are also involved. Double mutants of the sulphanilamide requiring strain and those with blocks to homocysteine synthesis grow if a little methionine is added. If more than a little is added they require sulphanilamide. ~~Thronine also antagonizes too~~ The sulphanilamide requiring strain will also grow when threonine is added. It appears that in the sulphanilamide requiring strain an aberrant reaction is taking place which uses up homocysteine and threonine, and is catalyzed by p-aminobenzoic acid ~~sulphanilamide~~ The details are complicated, and until the nature of the reaction catalyzed by p.-aminobenzoic acid is known, the matter will not be cleared up. p. 15a

I end with one example out of many possible where genetical work points ahead, just where we do not know. Houlahan and Mitchell (1948) examined trichloracetic acid extracts of Neurospora. They

determined phosphate immediately and after heating at 100° with 2N HCl for 10 minutes. Some extra phosphate appeared in all cases. Its source was in the mainly a polymetaphosphate whose Ba barium salt is insoluble in .1N HCl, perhaps a hexametaphosphate. It is hydrolysed by an enzyme from Neurospora. The Some was present in all strains, but the amount was increased up to twenty-fold in some mutants growing in minimal medium plus a minimal amount of supplement. The three mutants which gave the best yield were required pyrimidine, lysine, and nicotinamide. The latter (6:001) accumulated little when given nicotinamide or 3-hydroxy-anthranilic acid, but much when given indole, tryptophan, or hypoxanthine. A similar substance has been found in normal yeasts. It would appear to be a source of used in a variety of phosphor ylations, like adenosine-triphos-phoric acid, and may thus be very important. One may surmise that it plays a less important but perhaps essential part in heptose organisms. 16a

A few general remarks on this work may be made. From To a "classical" biochemist (which means in fact one who has accepted the point of view of Hopkins) this work must seem incomplete because in a rather few cases have the enzymes been isolated concerned been even approximately isolated, and they The s many intermediates are compared with yeast or mammalian muscle the systems concerned are ill understood. In particular it is likely that many phosphoric esters remain to be discovered. This As against this, wholly new fields of intermediary metabolism have been opened up. It is moreover possible that more is known than has been

Bonner, D. M., Tarnofsky, C., and Partridge C W H (1952) PNAS 38, 25.

published. Some of the workers concerned are now paid by the Atomic Energy Commission, and such an affiliation at best imposes a delay on publication, at worst holds it up indefinitely.

Bonner, Yanofsky, and Partridge (1952) worked with a number of the mutants blocking tryptophan and nicotinic acid metabolism described earlier. They found that 3401 which can use indole but not anthranilic acid for growth, can nevertheless grow without tryptophan when given nicotinic acid. What is more surprising, it can convert N^{15}-labelled anthranilic acid into tryptophan. They followed this up by making double mutants of 3416 (which produces quinolinic acid as a by-product) with C-83 (blocked between indole and tryptophan) 10575 (blocked between anthranilic acid and indole) and 25001 and 7655 (blocked before anthranilic acid). These double mutants were kept growing on unlabelled nicotinic acid, and given N^{15}-labelled tryptophan in the case of C-83, indole in that of 10575, and anthranilic acid in the other cases. In the case of C-83 all the tryptophan of the mycelium and the quinolinic acid of the medium had all been formed from the labelled precursor. In the other cases large amounts of both, upto 84% of the tryptophan and 71% of the quinolinic acid, had been made from other nitrogen sources. Further experiments showed that reversion of a gene and nitrogen atom exchange could not account for the observed fact.

Now C-83 lacks an enzyme, the other mutants were not known to lack one. They cannot metabolize one until they have started growing. This

Lenn J. and Lenn P. S. (1952). PNAS 38, 44.

~~Olitke, Tatum, Zubin, and~~ ~~J. Bc~~ C. 189, 429

Olitke, R. C, Tatum E. L., Zubin, J, and Block C. J. ~~Bc~~ (1951). J BC 189, 429

Lenn F. B. (1948). Genetics 33, 113

Among non-nitrogenous metabolites the most interesting work is perhaps that on "acetateless" mutants, of which there are at least three genetically different ones. All of them require acetate, ethanol, or perhaps other simple substances, if glucose is the sole carbon source. They grow slowly on glycerol as sole carbon source, but even a little glucose prevents such growth. It seems that some product of the normal metabolism of glucose inhibits the utilization of glycerol for acetate formation. Lein and Lein (1952) worked with a "suppressor" of "acetateless", that is to say a mutant which enables all three acetateless mutants to grow in presence of glucose, though slowly. In two cases the growth was at just the same rate as on glycerol only. It is suggested that the suppressor blocks the production from glucose of the substance which prevents the utilization of glycerol.

Oltha, Talin, Zabin, and Bloch (1951) used this same mutant, living on unlabelled glucose plus labelled acetate, to show that Neurospora synthesizes over 90% of its ergosterol from the labelled acetate and almost all its fatty acids from the labelled acetate. The methyl and carboxyl carbons are utilized in almost equal amounts. About 1% of the carbon in ergosterol can be derived from methyl carbons in isovalerate, whose previous conversion to acetate is not however completely excluded.

Lewis (1948) finds that "succinateless" mutants can utilize fumarate, malate, α-ketoglutarate, glutamate, and aspartate. It would seem that the tricarboxylic acid cycle may exist, but can be blocked at at least one point without lethal effect

phenomena, which the authors call "leakage", could be explained in many ways. For example 105-15 when ~~mutant~~ placed in a medium containing a limiting concentration of tryptophan, eventually grows to question like C-83. But ~~in this~~ for a week or so its growth rate increases until it may finally grow 10 times as fast. It appears to "learn" to make this substance.

The fact that a given metabolic step is blocked does not prove that this step is blocked. Nor however does it disprove the one gene - one enzyme hypothesis. The block may be caused by an inhibitory substance produced by, or not destroyed by, the enzyme controlled by the mutant gene causing the block.

~~The hypothesis that a metabolic~~

It is clear that we must abandon the hypothesis that every genetically determined block is due to ~~the~~ a failure to produce the catalyst concerned in the blocked process. ~~This however~~ A block can be due to inhibition by a metabolic product often chemically related to the substance which is not catalyzed, and probably to other causes. This does not however disprove the hypothesis that enzymes are direct gene products. The activity of enzyme δ on substrate A may be ~~also~~ blocked because enzyme β is abnormally active, producing an inhibitor B, or because enzyme γ, which normally converts B into something else, is absent or abnormally inactive, because the permeability of a membrane has been altered, and so on. It is still possible that δ is a primary gene product. Similarly in physiology the fact that the testis of an animal does not produce testosterone is adequate

Hershaham M. B. and Melchett H. R. (1947) PNAS 33, 223.

1, or perhaps two or three very closely linked genes,

Borek, E and Waelsch, H (1957). J. B C. 190, 191

Apart from "leakage", a good many examples of partial metabolic blocks have been recorded. Thus Houlahan and Mitchell (1944) described three allelomorphic mutants (in which the same normal gene has been altered). Their behaviour is summarized in Table 4.

Table 4

Mutant		Nucleotide need 25° C	Nucleotide need 35° C
37301 =	pyr-3[a]	3·3	3·15
37603 =	pyr-3[c]	0·38	2·3
37602 =	pyr-3[b]	0	2·4

Amounts of hydrolysed nucleic acid in gm/litre needed for half maximal growth.

—

Thus pyr-3[b] grows normally on minimal medium at 25° C but is lethal at 35° C. This may be due to a different temperature coefficient of a catalysis, to the more rapid heat inactivation of an enzyme, or even to a need for CO_2 in the medium. Bonner and Wilcox (1951) It is clear that genes with a gross lethal effects of this kind are more like those studied in other organisms than are the majority of those so far described in Neurospora.

, animals if the anterior pituitary, or even the eyes, are not performing their normal functions, in no way disproves the hypothesis that testosterone is formed by the testes. The analogy with diabetes mellitus (p.) is perhaps even more instructive.

fungi, mostly perhaps ascomycetes.

Chapter 4

Biochemical genetics of yeasts, bacteria, and viruses.

Yeasts are, of course, a specialized and morphologically degenerate group of ~~actinomycetes~~. ~~Most strains of yeast are haploid. However conjugation can often be induced, and in certain cases diploids can be propagated for some time without~~ meiosis. Sooner or later they usually give rise to haploids again. Some species are diploids in which the haploid generation is wholly or partly unable of independent existence. Unfortunately the cytology of yeasts is very difficult, and there is considerable controversy as to the genetical interpretation of observed facts (cf. Catcheside l. c.). ~~For this reason I~~

The cytology is very difficult. It is generally thought that most yeast cells (particularly those of the common baker's and brewer's yeasts, Saccharomyces cerevisiae) are diploid. They ~~or~~ can often be induced to give rise to four-spored asci, ~~from which~~. The spores may conjugate at once on germination or propagate themselves for some time as haploids. There are a good many inherited biochemical characters, but their genetics are a matter of controversy (cf. Catcheside l. c.). Winge, in Denmark, obtains results showing fairly clear Mendelian segregation. Lindegren and Spiegelman in America describe very irregular segregation, and transmission of acquired ability to ferment melibiose (cf. Chap. 7) for a thousand cell generations. Until this controversy is settled it is premature to ~~describe~~ draw far-reaching conclusions

It seems likely that some of the irregular segregation observed may be explained by polyploidy. Winge and Roberts (1950), who obtained good Mendelian segregations, attribute the results of others to the presence of several genes with like effects, and to overlap of generations. [2a]

The violence (whether justifiable posterity may decide) of the language used regarding yeast genetics can only be paralleled in connexion with the differences of opinion between Soviet workers and those of most other countries on general questions." — and his colleagues have come to presumptuous conclusions as a result of their superficial investigations" is a typical example. Fortunately No one who has not worked on yeast genetics can presume to judge in this matter. We can only be glad that it is not yet mixed up with politics!

from the genetical data. Biochemical mutants of the type described in the last chapter are known, e.g. but deletions to form different types of enzyme are necessary and here yeast offers a considerable technical advantage. If two biochemical mutants are known, one can plate out a mixture of them on a minimal medium. The only since abilities to make a substance or to utilize one are usually dominant, the surviving prototrophic cells are generally diploids with the normal dominant genes from both strains. Single cell cultures of these will segregate out forms with double biochemical deficiencies as haploids or double recessives.

In view of our extensive knowledge of the dynamical biochemistry of yeast, a really satisfactory technique for the genetical treatment of its biochemical mutants will be of the greatest importance. The work of Satyanarayana and his colleagues may point the way to important advances.

Bacterial genetics are difficult. Abnormal races are obtained by selection either by plating out individuals, usually after treatment which induces gene mutations in higher organisms, or by selection for and for resistance to a drug or a bacteriophage. In the latter case it is extremely hard to distinguish between adaptation and selection. Only in a very few strains is sexual reproduction known. In other cases we can say that two "mutants" with the same biochemical requirement (e.g. pyrimidine) are different if they grow together, but in a mixed culture, each producing a diffusible substance which supplements the other's needs; but we cannot state that the same gene has mutated if we cannot do so. Table 8 (after Catcheside

similarly chlorate resistant forms do not form CO_2 from sugars, apparently lacking carboxylase. The fact that the same mutation confers resistance both to chemical and physical mutagens strongly supports the view (see Chap. 7) that the action of radiation is fundamentally biochemical.

of a particular strain, K12, of E. coli

Lederberg ( ) has a list of some known markers in E. coli strain K12.

1. Nutritional needs. 12 of the usual amino-acids, and homocysteine.
Purines. Pyrimidines. 5 B vitamins. Sulphate. Sulphide.
2. Inabilities to ferment lactose and maltose.
3. Resistances to dichloracetate, to azide, and to lithium, and to sulphonamide.
4. Resistance to x-radiation and mustard gas (both at once).
5. Resistances to various strains of phage.

Some at least of the phage-resistant mutants also come under "aminoacidless" auxotrophs as they require tryptophan in their medium.

Multiple mutants are known. Thus Lederberg ( ) obtained a threonine-less form by X-radiation. Further X-radiation gave leucineless and threonineless, ultraviolet radiation removed the capacity to ferment lactic acid, and selective killing added resistance to a phage strain. Reversion to normal occurs by mutation and only of one gene at a time. When however two multiple auxotrophic mutant stocks are mixed, small numbers of prototrophs appear on interaction. Lederberg has made it reasonably certain that this occurs as a result of fusion of bacteria in pairs (never in triads) and that the genes show linkage as if they were arranged on a single chromosome. Lederberg (1949) has further obtained rather unstable diploid strains in which the normal characteristics are dominant. [3a]

But other inherited characters in bacteria are inherited in a different way. Pneumococcus can be classified into over 50 serological types on the basis of their capsular polysaccharides, which are antigens of very different compositions, e.g.

Avery, O.T., MacLeod C.M., and MacCarty M. J Exp Med 1944, 79, 137 (1944)

according to the results of Tatum, Drew and Pollard (1952) with deuteron bombardment.

Tatum D., Drew R., and Pollard E. R. (1952) PNAS 38, 180

Taylor H.E. (1949). Unités biologiques données de continuité génétique 45.

When grown on media not containing vertebrate sera, they "degenerate", and form rough colonies instead of the normal smooth ones. The capsular polysaccharides are lost, but ~~the smooth variants can~~ along with the virulence, but both can be regained by ~~them~~ in a suitable medium. When however a smooth form, derived, say from rough type II, is grown in suitable circumstances ~~and~~ in presence of a cell-free extract of Type III, it ~~turns into a~~ develops into a rough type III.

Avery (Mc Carty and Macleod (1944) ~~made it~~ purified the "transforming principle, and showed that it is a desoxyribonucleic acid, stable to proteases, amylases and ribonuclease, but destroyed by desoxyribonuclease. Hotchkiss (1949) describes the further purification. His best fractions had half the maximum activity at a concentration of $\frac{1}{2} \times 10^{-?}$, or, ~~since~~ the molecular weight is of the order of $\frac{6 \times 10^6}{2}$, at about 10^{-14} molar! Since there is no reason to suppose that even 1% of this nucleic acid has the ~~actual function~~ highly specific function of the transforming principle, the real concentration may be much less. The hydrolysate contains no uracil, but perhaps some 5-methyl-cytosine. (Taylor (1944) then carried the matter further. For transformation the ~~the~~ smooth bacteria must be held for about 5 hours with an agglutinating factor such as an antiserum, and another protein factor such as crystalline serum albumin. It is suggested that their function is to ~~extend~~ uncleic acid synthesis. Then 5 minutes' exposure with the transforming principle suffice. In 30 minutes about 1 Pneumococcus in 200 is transformed. Taylor has further worked with "extreme roughs" of Type III, and intermediates. She has shown that several "races" producing different amounts of ~~the~~ ~~same~~ substance at least antigenically the same polysaccharide produce qualitatively different

Bovin, ~~Ver~~ A, Vendrely R, and Lehoult Y. (1945) C.R. Ac. Sc. t4 221, 646

1 For a summary of work on these and other bacteria, see Boivin, Vendrely, et Tulasne (1949).

Boivin A, Vendrely, R, and Tulasne R. (1949) Les faits biologiques donnés de continuité génétique. 67.

What is much more important or remarkable, she has obtained crossing-over of transforming principles. That is to say by "infecting" Pneumococci with principles causing them to perform processes A and B separately, she has obtained a principle which causes them to perform both A and B, the two effects being now inseparable, or very nearly so.

transforming principles. Similar transforming principles will convert a rough type into a smooth type producing Type II or Type VI capsular polysaccharide. Bowen, Vendrely and Le Houte (1945) were able to transfer the capacity for making ~~bacteriophage~~ saccharase ~~and~~ from one strain of E. coli to another by a similar technique. Other workers had claimed similar transfers even between species. Thus (19) had produced luminous forms of Vibrio cholerae by growing them with

These claims have become quite plausible.

Let us be clear what has happened. A Pneumococcus takes up one or more molecules of nucleic acid of a certain pattern from solution. As a result it makes more of this particular type of nucleic acid, and, as do its descendants, and it also makes a particular kind of polysaccharide. Since the transforming principle is not destroyed by enzyme, it is unlikely that the nucleic acid contains the polysaccharide as a prosthetic group. When we further consider that chromosomes at some stages in their "life" cycle, consist of desoxyribose nucleic acid and very simple proteins, it seems reasonable to suppose that the transforming principle is not unlike a gene detached from its chromosome.

There may be yet a third type of reproductive "mechanism" in bacteria. Many types of bacteria are destroyed by phage, which multiplies 100-300 times in the process. A very few resistant mutants escape, and apparently do not harbour phage. There are however lysogenic strains of bacteria which harbour phage, ~~in~~ but only occasionally liberate it, in which case it can attack other bacteria. In some cases phage is only liberated in very special circumstances. That is to say two

Since this paragraph was written, Hays (1952b) calls attention to the fact that Weigle and Delbrück (1951) have shown that Lederberg's K12 strain harbours a virus liberated by ultraviolet irradiation, and discusses the possibility that the "genes" transferred in Lederberg's experiments are carried by, a part of, this virus, or phage. This is of course a return to older ideas that phage was part of the life cycle of bacteria. Until these questions are cleared up it is perhaps premature to homologize bacterial genes too closely with those of larger organisms.

Delbrück M. (1949). Unités biologiques douées de continuité génétique.
C.N.R.S. Paris. p. 91.

rate of reproduction of phage is almost exactly equal to that of the bacteria. Nevertheless in exceptional circumstances phage can pass from one bacterium to another. Here then is another case of transfer. A ~~phage~~ phage particle has a molecular weight of the order of 10^8 or 10^9, which is intermediate between that of a nucleic acid molecule and that of a chromosome.

~~The phage particle is~~ Phage bears its own genetics. Mutations occur, and as the result of a mixed infection phage particles are formed combining some of the characters of both their "parents". Estimates of the number of genes vary from about 20 to over 100. If this means that a phage contains only some 50 molecular species of nucleic desoxyribonucleic acid then separation becomes a task which would certainly be formidable, but ~~not so~~ incomparably easier simpler than the isolation of one of 10,000 or so genes from a metazoan chromosome, supposing each gene to be a separate molecular species. It is possible that the first genes to be isolated will be genes from bacteriophage. On the other hand the penetration of a transforming principle may be simpler. But it is perhaps in these simple organisms that the material bases of inheritance will first be specifiable in chemical terms.

Some of the mutant forms differ antigenically. Others require "activation" before they will attack a bacillus. Delbrück (1949) described races of T4 with different needs. One stock has no such needs. The normal type requires about 10^{-5} M L-tryptophan, or other substances in much larger concentrations. Another mutant requires tryptophan and Ca^{++}. Some of the tryptophan-requiring stocks are inhibited by small quantities of indole, others are not.

Again Davis (1952) has made it highly probable, from a study of mutants, that Escherichia coli ~~syn~~ forms lysine from L-ε-diaminopimelic acid, and not L-amino-adipic acid

Davis B.D. (1952) Nature 169, 534.

Comparatively little work has been done in using series of mutants to detect metabolic pathways in Bacteria, though doubtless a careful search of the literature would ~~enable us to~~ permit the compilation of relevant results. I therefore take ~~one~~ two examples to illustrate the lines on which such work is developing.

Plough, Miller and Berry (1951) worked on the needs of different mutants of Salmonella typhi murium. They report the following alternative needs :-

cystine / methionine	cysteine / serine	proline / glutamic acid	tyrosine / phenylalanine	tyrosine / tryptophan	adenine / guanine

and as regards sulphur metabolism, the needs shown in Table . The normal organism can obtain its sulphur from $Na_2S_2O_4$. None of the mutants listed in the table can do so.

Table 9

Mutant	Na_2S	DL-cysteine	L-cystathionine	DL-homocysteine	DL-methionine
A 141	+	+	+	+	+
B 1 M 0	+	+	+	+	−
C 2 0 3 1	−	+	+	+	+
D	−	+	+	+	−
E	−	−	+	+	+
F	−	−	−	+	+

It would seem that the metabolic pathway from cysteine to methionine can be traversed in both directions, whereas in Neurospora it appears to be a "one-way street" from methionine to cystine, in Neurospora from cystine to methionine. As however ~~doth~~ there is no technique for combining different mutants, conclu-

Pearcocke and Hutchelwood Sm C. (1948) Proc Roy Soc B .

-sions must be much more tentative than in the case of Neurospora and the history of Neurospora biochemical genetics shows how often mistaken conclusions have been drawn from data which could later be reversed by the combination of genetical and biochemical techniques. Except in the rare case where crossing is possible, this cannot be done with bacteria. Moreover such work as that of Peacocke and Hershelwood (1948.) shows that some of the effects of ultraviolet radiation are readily separable by transfer to a suitable medium under conditions which make an explanation by back mutation and selection extremely improbable.

There is no reason to expect a priori that the general principles of genetics should hold for bacteria. If some of them do so, that is very satisfying. But it seems equally unwise to argue, except in the most tentative way, from bacterial mutations to mutations in other organisms, or from non-Mendelian behaviour in bacteria against Mendelian behaviour in other organisms

Table 8

Some mutants of Escherichia coli

Requiring sulphite
" sulphide
" pantothenic acid
" p-aminobenzoic acid
" pyridoxin
" nicotinamide
" biotin
" thiamine
" threonine
" glutamic acid
" glutamine
" leucine
" isoleucine
" lysine
" proline
" arginine
" phenylalanine
" tyrosine
" tryptophan
" cystine
homocystine

Requiring methionine
Requiring purines
pyrimidines
Incapable of fermenting lactose
Resistant to lithium chloride
" " trichloracetate
" " ~~radiation and mustard~~ gas
Unable to ferment maltose
" " " lactose
Resistant to lithium chloride
" " dichloracetate - azide
" " dichloracetate
" " sulphonamide
" " radiations and to mustard gas
" " various strains of phage.

Hayes, W. (1952a) Nature 169, 118

" " (1952b) " 169, 1017

~~However~~ Lederberg's findings have been fully confirmed, and ~~the explanation of~~ this ~~discov~~ there can be no doubt of the importance of his discovery. But his interpretation of them ~~was at least open to question~~ Hayes (1952a, 1952b) ~~used two~~ as analogous to the sexual process in higher organisms is open to question. Hayes (1952a, 1952b) worked with two K12 strains, ~~W667~~ 58-161 which requires biotin and methionine, and W667 which requires arginine, threonine, and leucine. ~~If W667~~ They give prototrophic "recombinants" under Lederberg's conditions. If W667 has previously been treated with streptomycin or ultraviolet radiation in doses sufficient to "kill" it, that is to say to prevent it permanently from multiplying, no recombinants are produced. But such previous treatment of 58-161 does not stop the formation of recombinants, and ultraviolet radiation can even increase it upto 25 times. He concludes that 58-161 acts as a donor of nuclear material even when its cytoplasm has been so damaged as to prevent reproduction. W667 is a receptor (he avoids the words male and female) and the integrity of its cytoplasm is essential.

Winge O, and Roberts C (1952). C.R. Lab. Carlsberg, 25, 141

Their most recent work (Wenge and Roberts 1954) deals with the segregation of the following genes.

R_1, R_2, R_3. Each produces a β-fructofuranosidase, permitting the fermentation of sucrose and raffinose, adaptation requiring about 5 hours or less

M_1 Produces an α-glucopyranosidase, and can ferment maltose or ~~glucose~~ sucrose, but after one or more days.

M_2 produces a specific maltase which does not allow the fermentation of sucrose, even after 14 days' training on 1% glucose + 1% sucrose

M_3 and M_4 produce a maltase of some kind, but have not yet been obtained without any of the others, ~~so it is not clear~~ the specificity of the enzyme produced by them is not clear.

M_1 and R_1 are very closely linked, with about 1% recombination, which gives $m_1 r_1$ cells which will not ferment sucrose.

It is however to be noted that among 2241 ~~22~~ asci from which 964 ~~908~~ spores were isolated and followed up, the authors find it necessary to postulate no less than three mutations affecting the rather few genes with which they were working. Rates of this order are pretty well unknown elsewhere except for the "multimutating" genes which cause flaking in flowers. It may therefore be that their interpretation is not final.

Reeman, G. H. (1931). J. Agric. Res. 42, 5

Protocatechuic acid can be derived from cyanidin and quercetin with strong alkalies, and may be their metabolic precursor.

Chapter 5

Some Biochemical genetics of higher plants

Numerous colour varieties of a great many cultivated plants are in existence, and their genetics have been investigated. Often the differences are confined to the flowers, ~~but their~~ and flower colour has been more extensively studied than that of other organs. However the stems and leaves generally contain the same anthocyanins as the flowers, though in smaller amounts, and thus may be of economic importance, as in onions, where Riemann (1931) found that coloured varieties are ~~comparatively~~ resistant to the fungus Colletotrichum circinans ~~to~~ apparently because of their protocatechuic acid content. This chapter was somewhat sad one for me. In 1932 I left the Department of Biochemistry at Cambridge, with a promise that I should be given facilities for the study of biochemical genetics at the John Innes Horticultural Institution. I had hoped, in particular, first to investigate the enzymes concerned, ~~first~~ in pigment production, and later those concerned in carbohydrate metabolism. For reasons ~~reasons this~~ promise was not kept, and I found that I was cut off from biochemical research. Doone has in fact attacked the problems in question, and it was left to Beadle's school to do similar work on simpler organisms.

Flower pigments are almost all of two types. Some flowers have plastid pigments, yellow ether-soluble carotenoids and often a little chlorophyll. The plastids may be absent as in the rose, present in a central "eye" as in the primrose, or present throughout the petal, as in many Cruciferae. All flowers also

possess water-soluble pigments of two related types. The anthocyanins absorb in the various parts of the visible spectrum, and vary in colour from red through purple to blue. The anthoxanthins, which may be flavonols, flavones or chalcones (see Fig) absorb in the violet or near ultraviolet, and are yellow or white. However all of them are coloured to bees, or more accurately the bees react differently to an object coloured by a "white" anthoxanthin which absorbs in the near ultraviolet, than to an object such as white paper which does not do so. The production of anthocyanins and anthoxanthins is controlled in part at least by the same genes. That of plastid pigments is controlled by different genes. Each class of gene may also have structural effects. Thus a recessive gene for large "eye" in Primula sinensis gives crimped leaves and petals, one for "hooded" standards (petals) in Lathyrus odoratus. To get a very dark flower such as the brown wallflower (Cheiranthus cheiri) both types of pigment can be used, so that light is absorbed over most of the visible spectrum. ~~I should however say~~ Very little is however known as to the biochemical genetics of plastid pigments.

The anthocyanins always, and the anthoxanthins usually, exist as glycosides. The Fig . gives the constitution of the aglucone residues. ~~Occas~~ The sugars, which may be monoses or bioses are attached in the 3 or both the 3 and 5 positions. Both types of pigments are indicators. The anthoxanthins become yellower or yellower in alkaline solution, the anthocyanins are ~~blue~~ red at pH 2-5, often blue at 7-10, and may be purple or colourless in between. However pelargonidin derivatives do not turn blue. Various ~~organic~~ colourless organic copigments also affect their colours. At a given pH the round 5 the more hydroxyls are present, the either on the agla-

Scott-Moncrieff, R. (1939) Ergeb. Enzymforsch. 8, 249

The blue or albumine condition is recessive

Lauer sucs (1950) account of statement to the contrary is incorrect

anthocyanidin or (though this is much less important) on the sugars, the bluer is the colour. Salmon-pink flowers usually contain pelargonidin derivatives, the most vivid blues delphinidin derivatives. They are present in large amounts, up to 10% of or more of the petal dry weight, and their capacity for crystallization makes them far easier to study than most animal pigments.

Flowers of very different colours may contain the same anthocyanin. If so an anthocyanin solution at the pH of the ~~wild~~ cell sap of the wild plant is usually red, the wild type flower is usually purple, red being due to a recessive gene, and bluer to a different recessive. At an early stage in her work Scott-Moncrieff (1939) ~~had great difficulty in~~ needed great tact to collaborate simultaneously with Sir Robert Robinson and myself. He maintained that bluer was due to organic substances, I was sure that it was due to ~~pH~~ higher pH. Both turned out to be right. The sap of blue or purple flowers always contains a copigment, usually a colourless anthoxanthin, which forms a labile purple compound with anthocyanin. This compound is reversibly dissociated eg boiling, or the anthoxanthin can be extracted with ethyl acetate. In red flowers a recessive gene ~~a~~ blocks the synthesis of anthoxanthin, and usually leads to an increased production of anthocyanin from other common precursor. In blues (~~bb~~, so far always recessive to purple) the pH of the petal sap, though not that of the leaves or stem, is increased by about .6 pH. Thus using a glass electrode, Scott-Moncrieff found a pH of about 5.3 in purple and red ~~primulas~~ Primula sinensis, and of 6.0 in blues. The alkaline red form is usually a rather dirty purple, and seldom grown commercially.

Lawrence N. J. C. (1950). Brookhaven Sym. Symp. 4, 3

for a 3-bromide (hexose-pentoside)

In white or yellow flowers there is no anthocyanin. This is usually due to a recessive gene blocking synthesis, sometimes to a dominant inhibitor. In a few most white forms the anthoxanthin synthesis is not blocked, on the contrary more than usual is formed. In a few recessive whites (e.g. in Antirrhinum and maize and Pharbitis nil) both anthocyanin and anthoxanthin formation are blocked. Such plants are generally rather feeble. Similarly in the seeds where anthoxanthin synthesis is blocked there is usually more anthocyanin than in the purples where both types of compound are formed. Genes are frequently found which regulate the amount of these compounds without suppressing their formation.

Some of the colour differences between domestic varieties are due to differences in oxidation in the 3' and 5' positions, others, generally smaller, to changes in the sugars attached to the main molecule, ~~that is to~~ or to methylation of the hydroxyls. The more oxidized forms are usually, if not always, dominant over the less oxidized, the methylated at least sometimes over the demethylated. Less is known about the genetics of the sugar residues. In Streptocarpus Lawrence (1930) found that the gene D generally ~~converts a 3-~~ substitutes ~~glucoside (hexose pentose)~~ into a 3-;5-, diglucoside, but his results suggest that the second hexose cannot be attached to a pelargonidin residue, so a D plant with D and the two recessive genes for pelargonidin has a pelargonidin 3-hexoside

Since both anthocyanins and anthoxanthins can be oxidized in the 3' position by dominant genes, it might be supposed that the same gene would be responsible for both. This is not so in Antirrhinum majus where one dominant gene substitutes cyanidin for pelargonidin, another substitutes the

yellow luteolin for the ~~colo~~ ivory apigenin. In Primula sinensis the kk (pelargonidin) plants are certainly more liable to fungal attacks and probably weaker in other ways than those ~~aa~~ containing cyanidin, which is hard to understand if their metabolism is the same except as regards this one molecule.

The biochemical genetics of flower colour is best known in the sweet pea Lathyrus odoratus (——————). The wild type has purple flowers, the two lateral petals being lighter and bluer than the others. Almost all horticultural varieties are homozygous for a recessive gene ~~dep~~, and the petals have a more uniform deep colour. ~~The other colour genes found in cultivated forms are all~~ The anthocyanin is mainly malvidin 3:5- ~~mono~~diside, the anthocyanin quercetin, ~~but~~ which is colourless but a cofigment ~~smaller~~ amounts of other ~~&~~ related substances are found. The recessive genes in cultivated forms are as follows.

1. ~~Co~~mpletely blocking anthocyanin formation Gr & c, r, partially blocking it, co, p. The latter genes produce flowers with little anthocyanin except on the petal borders
2. Completely blocking anthocyanin ~~production~~ formation ~~Ki, K, partially~~ m, k, partially blocking it, b, (and Dv), ~~bl~~ partially blocking it in co co. plants, &c.
3. Substituting ~~pro~~ for pelargonidin (one ~~methoxyl~~ methoxyl) ~~sm~~ sm, substituting malvidin for pelargonidin (two methoxyls), ~~Bl~~ e. sm only acts in
3. Substituting peonidin for pelargonidin (one methoxyl) ~~B~~, substituting pelargonidin for peonidin or malvidin, sm.
4. Raising petal ~~p~~ H from 5·34 to 5·93 (averages) ~~de~~

Although no genes are known blocking synthesis of both anthocyanins

Lawrence, W. J. C., and Pease J. R. (1940). Biol. Rev 15, 35

Haldane (1941). New Paths in Genetics. London.

As I devoted 29 pages of this book to the general genetics of anthocyanins, I dealt with the question in a more summary manner here. . .

and anthoxanthins are known in this species, the action of these genes clearly shows that they have a common source. mm and kk (maroon) and mm ee and kk ee (red) flowers have more anthocyanin than the corresponding copigmented types, co co and pp flowers contain abnormally large amounts of copigment.

There are also unanalysed but hereditary differences in methylation, and some varieties have a considerable amount of a 3-glycoside.

Every plant so far investigated has slightly different flower colour genetics. Where the wild type is red, anthoxanthin may be absent, as in the common poppy Papaver rhoeas (where it is only found in the dark basal petal spot). Here the presence of anthoxanthin is recessive to its absence. While ~~pelargonidin is usually dominant~~ the complete replacement of more oxidized forms by pelargonidin is always recessive (as if an oxidation were blocked), one dominant gene in Primula sinensis and two in Papaver rhoeas cause formation of pelargonidin along with more oxidized forms. Fuller details and references were given by Scott-Moncrieff (1932), Lawrence and Price (1940), and Haldane (1941).

But one point may be noted here. A number of genes, usually recessive, suppress anthocyanin formation locally. Thus in Primula sinensis the wild type has anthocyanin in the flowers, the axillae, the pods, and the seed coats. The white flowered types are due to a recessive gene which blocks anthocyanin formation throughout the plant. Other, different, recessives, block it locally in the axillae, pods, and seed coats. Similarly in Primula sinensis a dominant gene suppresses anthocyanin formation in the petals. A recessive is rarely present in the stems and leaves. Most of the other genes affect it through-

Until such work has been done, it is surely a mistake to write, as Lawrence (1950) does, ~~of~~ "of the conversion of one anthocyanin (~~calendin~~ malvidin) into another by the action of a single, dominant gene" (malvidin 3 desmoroside) into another (pelargonidin desmoroside) by a single dominant gene". There is not the faintest evidence that the malvidin desmoside is ever produced and then demethoxylated. If such an event occurs at any one of to the anthocyanin, the anthocyanidin, or a precursor.

where it is of economic importance.

- out the plant. Thus one changes can produce local chemical differentiation. I know, however of no case of a gene which alters the chemical nature of the anthocyanin in one part but not another, of a plant, though they may exist.

I [illegible] perhaps now the whole suggestions ~~some lines of research which I had hoped to carry out myself~~. As very little ~~work~~ research has been done in this field in the last twelve years it may be worth setting out some of the problems ~~on which~~ I had hoped to carry out or direct research.

1. A search for enzymes responsible for biochemical differences, e.g. a polyphenol oxidase, and for inhibitors of enzymes. Thus one would look for the absence of an enzyme in a recessive white, for the presence of an inhibitor in a dominant white.

2. A full investigation of the sugars of anthocyanins and anthocyanins. Are they correlated with other glycosides, or with carbohydrates in the same plant?

3. Further work on methylation, and an attempt to link it up with the metabolism of methionine or betaines.

4. ~~Attempts to alter colour of flower by grafting~~ Investigation of the origin of pH differences (with simultaneous work on the same question in fruits)

5. Attempts to alter flower colour by ~~injecting~~ (a) possible selective enzyme inhibitors, and (b) possible missing metabolites.

6. These would lead up to an attempt to ~~give a full~~ account of anthocyanin formation ~~as is that of nicotinamide formation in Neurospora~~.

7. Once the nature of the chemical process controlled by genes were known, an attempt to explain their morphology and effects where these exist.

Bridel, M., and Bourdouil, C. (1932). Bull. Soc. chim. biol. 14, 214

Terroret (M.) M. G. (1935). Bull. Soc. Chim. Biol. 17, 1235

Stage 4 is one where the pod is first oval, at stage 6 it is yellow. In the The round peas above were studied in earlier stages. They contained some sucrose and starch, but no stachyose. Reducing sugar (? glucose) was present in amounts always less than 1 gm./kilo, and disappeared completely in the dry round peas, while 0·15 gm./kilo remained in the wrinkled. The amounts of sucrose and stachyose in the dried round peas are unknown, since it contains some substance to give a reducing sugar with emulsin other than stachyose or mannose or fructose

8. A thorough investigation of gene controlled ~~differ~~ chemical differentiation.
9. The introduction of a uniform nomenclature for genes controlling similar processes in different plants.

A good deal is known as to genes controlling differences in the carbohydrates, especially the reserve polysaccharides. The first to be investigated genetically was Mendel's recessive gene wrinkled ~~sty~~ cotyledon in Pisum sativum. This was shown by Bridel and Bourdouil (1932) and Tomoi Tamari (1935) to substitute stachyose for starch. Table (after Bridel and Bourdouil) suggests that in the wrinkled form there is a partial block to starch

Table 10

Stage	Round			Wrinkled		
	Sucrose	Stachyose	Starch	Sucrose	Stachyose	Starch
IV	4·83	0	11·5	2·4	0·3	3·2
V	0·8	1·4	16·8	3·0	0·6	4·3
VI	0·2	2·5	20·6	2·4	1·0	4·2
dry	?0	?<6.0	34·6	2·2	11·4	14·8

Quantities in gm/kilo ~~dry weight~~.

—

~~catalyse~~ hive glucose and hive galactose residues

synthesis, so that a good deal of stachyose (a tetrasaccharide) and a little sucrose accumulate. It would clearly be of the greatest interest to investigate the enzymes, particularly the phosphorylases

Cameron, , and Tesa, (19) PNAS.

and curiously enough agrees much better with the visual judgment of the nature of the fire than does the stimulus content.

and starch-forming enzymes of these varieties, and to compare the starches by modern methods. Some at least of the starch in normal kernels seems to originate from the galactose residues of stachyose, but the conversion of one hexose to the other can hardly be the only process involved.

The work of Cameron and Teas (19) on Zea mays shows how complicated the situation may well be. In maize the endosperm is a triploid tissue, receiving two sets of maternal and one of paternal genes. The same genes, which in the haploid condition determine starch in the triploid condition, determine the nature of the polysaccharide of the endosperm, as in the haploid condition or that of the pollen grains, the two pairs of reserve products staining differently with iodine. As judged by eye, at least one of two dominant genes Su and Du were needed to produce a starchy endosperm with a smooth seed, and though the total carbohydrate is roughly constant, the sugar content may rise sharply at the dividing line. The nicotinic acid was estimated by biological assay. Preliminary results showed that amounts varied even more, from .18 to .98 $\times 10^{-6}$, and proteins in the same duration, and proteins had a similar trend. These results show clearly that the different types of carbohydrate are indicative of far reaching biochemical differences which will require very thorough investigation.

Earlier results of Mangelsdorf and Fraps ( ) show that the nicotinic content of the endosperm varies fairly directly with the number of genes at each of the loci. A recent finding in maize is a fluorescent mutant produced by the Bikini atomic explosion (19). The

Table II

Number of Sugars	Number of Diglycosides	% of starch	nicotinic acid $\times 10^6$
3	3	80.4	21.4
3	2	44.4	22.3
2	3	76.0	21.4
2	2	74.6	23.5
3	1	42.4	22.4
1	3	69.7	22.2
2	1	41.8	24.4
1	2	40.3	25.5
1	1	58.6	24.4
3	0	51.5	44.3
2	0	42.0	46.2
1	0	33.6	51.6
0	3	32.2	56.3
0	2	30.0	48.2
0	1	24.2	50.4
0	0	14.2	56.7

Composition of dried endosperm

Mangelsdorf P. and Fraps G. (1931) Science 73, 241

as to the interpretation of such results

West, F. W, Rosen, A. L, and Zechmeister L. Plant Physiol 17, 41

Mackinney G. and Jenkins J. A. P.N.A.S. 38, 48

fluorescence is due to anthranilic acid, which is present in about 1000 times the normal concentration.

A little is known of lupine metabolism. In maize Mangelsdorf and Fraps (1931) found that the yellow colour of the endosperm was mainly due to zeaxanthin, and proportional to the number of Y genes in the endosperm. The same is true for β-carotene (Table 12).

Table 12

Number of Y genes	β-carotene × 10⁶
3	4·50
2	3·00
1	1·35
0	0·03

The sharp proportionality should act as a warning! It certainly does not indicate that zeaxanthin and carotene are primary gene products. It does suggest that we are dealing with a process in which the amount of catalyst formed by the gene Y is a limiting factor.

In the tomato (Lycopersicum esculentum) Went, Rosen and Tschermezian (1942) stated that the gene R formed flesh caused the appearance of lycopenes, which are absent in r r fruits, and increased the amount of carotin about tenfold. Mackenney and Jenkins (1952) carried the analysis further. Lycopene includes a group of isomers, and pro-lycopene. They found that R R and R r plants make more carotenes and more phytofluene than r r. But a recessive gene t increases the amount of carotenes at the expense of lycopenes in R R and R r

1) mainly brolyscope

plants, but allow the synthesis of an appreciable amount of lycopene in ^ plants, along with a little phytofluene. ~~They suggest that the gene T is involved in the dehydrogenation of carotenes to form lycopene.~~ In particular R R tt plants make γ and ψ-carotenes which are almost if not quite absent in other genotypes. The details given are most interesting, but it is ~~hard to~~ not yet possible to state what processes are controlled by these genes.

Waldron, S.G., Abegg, F.A., Elder, J.A. and Hendricks S.B. (1946). Arch. Biochem. 10, 141

An equally interesting problem is that of self-sterility. In many plants there is a series of alleloms as S^1, S^2, S^3, etc, such that pollen tubes carrying S^1 cannot grow in the styles of plants carrying the same gene, such as S^1S^2 or S^1S^3. For fertilization to occur, the pollen tube must carry a "foreign" gene, and presumably a "foreign" gene product, the exact opposite of the situation as regards tissue grafts in higher animals. It is tempting to suppose that we are dealing concerned with colloidal gene products of the same kind of molecular size as the antigens. If so, a single species can produce any of over fifty different ones

Cryptostegia grandiflora forms rubber, while C. madagascariensis accumulates an ester of the triterpene lupeol. Bobbin Weldman, Abegg, Felder, and Hendricks found (1946) found that rubber formation was dominant, and attributed the difference to a single gene.

Finally

found that in a recessive fluorescent maize mutant induced by the Bikini atom bomb, the fluorescence is due to anthranilic acid, which is present in about 1000 times the normal concentration. As the plants are apparently healthy, either the block to tryptophan synthesis from this substance (assuming a pathway similar to that in Neurospora) is not wholly blocked, or there is an alternative pathway.

A large number of lethal and semi-lethal chlorophyll-less mutants have been studied. In Zea Mays chloroplast formation requires the co-presence of at least 15 autosomal genes loci; and genes at perhaps as many more are needed for full greenness. The homozygous recessives die, though they can often be kept alive on sugar. But they grow little if their reserves are exhausted, so there is little wrong with their general metabolism. Thus at least suggests that chlorophyll synthesis is a very complex process. Attempts to elucidate the biochemical differences between different chlorophyll-less mutants have met with small success.

One of the greatest gaps in our knowledge relates to the odours of flowers. These have a precise biochemical basis. They are very variable between species, and sometimes within a species. They are of very great biological importance in attracting insects, and therefore of evolutionary importance. But they are in some cases economically important. But as a field for biochemical and genetical research they have been greatly neglected.

for different reasons. Finally

Chap 6.

Biochemical genetics of

~~The Genetically determined metabolic differences~~ higher animals, especially man.

Since many most colour differences are biochemical differences though a few are structural, the data of elementary genetics contain, implicitly, a great deal of biochemical information. Until however the nature of the genes is known, little biochemists can learn little from such data. In consequence ~~about~~ a large fraction of all the ~~two~~ data on animal biochemical genetics refer to are based on the study of human biochemical abnormalities. These have been discovered ~~frankly~~ because they gave rise to pain, weakness, and death, like cystinuria or phenylketonuria, ~~be~~ secondly because they produced almost harmless but obvious abnormality, like the blackening of the urine of alcaptonuria. Thirdly because they simulated the biochemical symptoms of more serious conditions, like pentosuria. Fourthly, like β-aminoisobutyric aciduria they have been found in the course of laboratory work in apparently normal people. Family investigations (cf Harris 1953) have then disclosed their genetic basis.

The few data which we possess on insect biochemical genetics were mostly arrived at as follows. In Drosophila melanogaster and other insects mosaics are occasionally produced in which different parts of the body have different genetic compositions. The commonest cause is the elimination ~~in part of the body of~~ one of at an early cleavage division of the sex-determining X chromosome, giving insects in which some tissues contain two such chromosomes, ~~thus~~ and are female, others only

Caspersi, [19) Advances in Genetics 1,

one, so that they are rule. These tissues develop almost independently. Instead of sex hormones penetrating the whole body as in vertebrates, they can (at least in Habrobracon juglandis) diffuse through a few cells only. The same is true of most other biochemical characters. ~~Many of the~~ There are for example sex-linked recessive genes for white eye and yellow cuticle. If the chromosome containing their normal dominant allelomorphs is lost, as may happen in a Drosophila, the normal dark red, the other white or buff white, patches of cuticle may be yellow, and similarly for most other genes. This does not hold for a few genes, notably the sex-linked recessive vermilion, which suppresses the yellow pigment of the eye, leaving a scarlet. Here the two eyes of a fly are always of the same colour. Further an eye mutant from a vermilion stock transplanted into a normal larva develops the normal colour, and a normal testis transplanted into a vermilion larva will restore normal coloration to the eyes of the imago derived from it. Clearly the same normal tissues produce a diffusible substance which can be utilized by ~~the eyes of~~ eyes not containing the normal allelomorph of vermilion to make pigment. Another recessive cinnabar behaves in the same way. As the result of extensive work by Beadle, F. Ephrussi, Kikkawa, Butenandt, Wendel, and many others, summarized by Custori ( ) it turns out that the yellow pigment missing in vermilion, cinnabar, and similar eyes is a group of ommatins (Becker 19) dyes consisting of a tryptophan derivative attached to various proteins. The diffusible substances are kynurenine and 3-hydroxy-kynurenine, the blocks in vermilion and cinnabar being between

Butenandt, A., Weidel W., Weinhut R., and Dergunow W. Zeit. [illegible] Chem. 249, 229

Green M. M. (1947). Genetics 34, 564.

Butenandt A, and Albrecht W. (1942) Zeit. Naturforsch. 7b, 284

of tryptophan

and short of tryptophan and

tryptophan and kynurenine, kynurenic and 3-hydroxykynurenin (Balenandt, Wendt, Weichert and Dorjugin (1943). Free tryptophan accumulates in vermilion Drosophilae (Gren 1943)

Similar mutants are known in other insects. Caspari (1941 and earlier) worked with the moth Ephestia Kuhniella. This has a recessive mutant blocking ommatin synthesis at the same point as in vermilion in Drosophila. The eyes contain more tryptophan than normal, both in their non-protein nitrogen and their protein nitrogen (Balenandt and Albrecht 1952) and some of their proteins appear to be antigenically different from the normal. Rendtin and Schultz (19) devised a minimal medium for Drosophila melanogaster which contains various amino-acids, including tryptophan. When the amount falls below a certain limit there is considerable larval mortality, but the survivors produce more or less vermilion-eyed flies. On such a medium genetically vermilion flies survive better than normal. They do not "waste" the small available amounts of tryptophan by using in making non-essential pigments.

This is quite analogous to Macdonald and Ponteconvo's (p.) findings and as a means by which unused biochemical capacities may be lost as a result of natural selection rather than a Lamarckian process. ~~In a~~ For as a population containing vermilion genes these would spread, so that the failure to produce ommatin ~~or~~ normally namely due to the diet would become genetically fixed.

13a (after 11)

Many pigmentary mutants are known in birds. Their genetics and biochemistry are completely different. Thus the feathers of the normal budgerigar (Melopsittacus undulatus) are mostly green. They contain melanin and an ether-

The Beauceron melanic, ~~the pigment~~ a fowls legs may or may not have yellow pigment. On dissection the yellow-legged fowls are found to have yellow pigment in all their fat. The pigment appears to be xanthophyll, and is inherited by a dominant gene acting in the same way as that of rabbits described in Chap I.

soluble pigment. ~~Some~~ One recessive blocks the formation of the yellow pigment, giving blue birds, the blue colour being apparently "structural". Several others block melanin formation, giving yellow birds with ~~eyes~~ normal red eyes. A white bird is always a double recessive; which has lost both either soluble pigment and melanin. On the other hand in the domestic fowl (Gallus domesticus) the yellow feather pigment is a "phaeomelanin" derived from aromatic amino-acids. The genetical control of pigment is not unlike that of the water-soluble flower pigments. Some genotypes ~~have no yellow~~, like the Light Sussex and Cuckoo Leghorn, give ~~us~~ ye black pigment but no yellow, others like the Pile bantams have yellow and no black. In the dominant whites ~~both~~ the formation of both pigments is inhibited by independent genes, but in the several different types of recessive white the formation of both pigments is blocked, and the genetical evidence makes it clear that they are also biochemically related.

In mammals, although the ~~in~~ exact composition of the hair pigments is not known, a good deal of granditative work has been done, namely by Wright ( ), Russell ( ) and Russell and Russell (19). ~~In mice there are genes affecting colour at~~ different loci. There appear to be three chemically different types of pigment, namely yellow, brown and black, but brown and black are probably alternatives, only one being formed in a given animal. Colour differences depend both on the amounts of these pigments and their arrangements, both on the coat as a whole and within individual hairs. ~~At least~~ 24 genes of mice are known which affect the coat colour (Grüneberg 1952). ~~Of these~~ Genes which affect the colour of mouse hairs are known at at least 24 different loci (Grüneberg 1952). At eight of these loci there are genes with

which also lightens the brown colours

Not all these genes are concerned at all directly in pigment formation. Thus "blue" mice differs from black in having larger pigment granules, and hence a smaller absorbing surface, but may have rather more black and yellow pigment. Perhaps they lack a surface-active substance which breaks up large granules. It is intelligible that such a biochemical difference may also be responsible for their slightly longer bones. Again the genes for piebaldness probably inhibit the migration of pigment-forming cells from the neural crest. I am inclined to doubt whether much more than ½ of the mouse colour genes (a, b, c, gl, mi, p, pa and rst) are directly concerned in melanin pigment synthesis.

A further point is important. Among the few genetic differences between related subspecies and species which have been isolated are rather small changes in the activity of some of these colour genes, doubtless reflecting deeper metabolic changes in metabolism. We are therefore studying biochemical evolution as well as biochemical variation.

highly specific effects on other characters. Some of the remainder have measurable but non-specific effects on growth. Two of the colour mutants (one causing absence of all pigments, the other absence of yellow only) also cause insufficiency of the osteoclasts, leading to characteristic skeletal abnormalities. One causes a macrocytic, rather a microcytic anaemia. Va causes gross disturbances of locomotion, pa minor disturbances due to the absence of otoliths. Spl causes prenatal death with spina bifida, and A^y prenatal death before implantation. Clearly many of these genes, like the gene for phenylketonuria in man, affect other processes more important than pigment formation. A^y when heterozygous has a huge effect on weight, by encouraging fat formation. Females increase in weight by 62%, but their body length increases by 5%. Other colour genes: Some other colour genes increase or decrease body length by 1–3%, and may do so even when heterozygous and with no visible effect on the hair colour. It is clear then that if the biochemical genetics of hair pigmentation fully, we should also have information on the developmental biochemistry of the bones, blood, and nervous system. Since two different genes affect the osteoclasts, it is clear that these cells must share some metabolic peculiarities with the hair-forming cells. On the other hand pigment formation is not such as correlevant. Albinos (c c) do not differ measurably from normals in weight or viability under laboratory conditions.

The most complete genetical work (surprisingly little is known about the chemistry of melanin formation in different genotypes. Normal mouse skins possess an insoluble (or difficultly soluble) enzyme, dopa-oxidase, whose amount, or activity can be measured by the rate at which it produces black pigment from a "dopa"

3-4-dioxyphenylalanine. This enzyme is absent in the white parts of piebald mice, and in albinos. Its activity is much reduced ~~in some~~ by some ~~non-black~~ allelomorphs of C, namely c^{ch}, c^{a} and their heterozygotes with one another and C (Russell and Russell 1948). ~~Boot~~ This reduction runs parallel with a reduction of yellow pigment (phaeomelanin) but not of black or brown pigment (eumelanin). Whatever dopa-oxidase is doing in ~~mouse~~ skin, it is not oxidizing 3-4-dioxyphenylalanine, though it is very probably concerned in some phase of the production of yellow pigment from a ~~colourless~~ precursor. ^ 6a

Let us now turn to Wright's granddaughter work. He hydrolysed defatted guinea-pig (Cavia porcellus) hair in boiling HCl, removed the chloride, boiled with .2 N KOH, and extracted pigment colorimetrically. The standard error of a single sample was 3-5%, but groups of 20 gave quite accurate results. He worked mainly with non-agouti animals that is to say with uniform rather than banded hairs, using the following genes

C, c^k, c^d, c^r, c_a^a, all allelomorphs, the lowest being almost white

E, e. ee animals have practically no black or brown stain in hair pigment

P, p. p dilutes hair colour, and gives a pink eye

F, f. ff animals have dilute hair colour at birth, which fades further with age

B, b. bb animals have brown pigment instead of black.

These genes give 12 15 genotypes, ~~not all distinguishable, often allowing for dominance~~ but the number distinguishable is much less both because of dominance and because many are nearly white. The C alleles have quite different effects on yellow-pigment and on black with brown. For yellow the order is

and for brown coat $c^R = 82$ $c^* = 63$, $c^d = 63$, $c_a = 0$ for brown

one being a derivative of tyrosine, the other of tryptophan.

$C > c^k = c^d > c^v = c^a$ ∞. If $C = 100$, $c^k = 20$, $c^d = c^d = 20$, $c_v = c_a = 0$,
for black ~~and brown~~

$C > c^k > c^v \geq c^d > c^a$ ∞. If $C = 100$, $c^k = 41$, $c^{dv} = 42$, $c^d = 34$, $c_a = 0$ ~~for black~~,

In each case c^a produces no appreciable amount of pigment. These results are most easily explained if each allelomorph produces a different enzyme an enzyme, and these enzymes differ in their relative specificities as regards the precursors of the two types of pigment; ~~The effects of a pair of genes are only additive and they~~ ~~form small amounts of pigment, while when they are together, along with the mature gene c^a for~~ ~~larger amounts the effects of the two together are less than the sum. This is just what we~~ ~~should expect if the enzymes were competing for substrate.~~ The figures given are amounts of pigment produced by one gene along with c^a. Two active genes together produce an additive effect if each has a small effect, and somewhat less than additive, e.g. $C^k C^d = 80$ for black pigment, if each has a larger effect. This is what we should expect if the immediate effects of the enzymes were additive but their products were later transformed by another enzyme with a Michaelis constant. P and F control other stages in the formation of melanin. Remarkably, P P bb produces less brown pigment than P P b b in presence of C. This suggests that one of the enzymes concerned has an optimum substrate concentration, like many lipases and some oxidases, and can be inhibited by excess of substrate.

Wright's papers must be read for a detailed account of these interactions. They leave no doubt that the biochemical account will show be extremely complicated. The amounts of pigment produced by various genotypes depends on their age and temperature. Some genotypes darken with age.

these enzymes + other than on the ~~sup~~ amount of substrate available for them.
Kalmus, H (1941) Proc Roy Soc B, 130, 185

~~position discussed in Chapter~~ on p.

These genes, discussed later, cause phenylketonuria and alcaptonuria. Phenylketonurics, who cannot oxidize phenylalanine and related compounds in the para position, have light hair. Alcaptonurics, who cannot oxidize homogentisic acid, not only produce a urine which blackens on standing, but for a pigment in their cartilages, ~~Their hair~~ colour ~~may be by a process~~ which may become hard and brittle. The analogy with insect antisender pigmentation is obvious.

~~due to the failure to oxidize phenylalanine and related compounds in the para position~~

others aside. The full account will include the biochemical action of all the colour genes. ↗ 8a (in Vol 2)

The cuticular pigment of insects is due to the action of ~~local~~ enzymes on the cuticle on phenolic acid found in the haemolymph, and the differences due to various genes (e.g. yellow and ebony in Drosophila melanogaster) seem to depend on ↗ ~~enzymes in the cuticle~~. Hadorn (1941) showed that the darker cuticles are less permeable to water and other substances than the lighter ones. Thus yellow flies lose water quicker than brown and black ones in dry air, and after drying over sulphuric acid regain water quicker when replaced in normal air. The skin colour of insects may therefore be adapted to differences of humidity as well as to concealment from enemies and other forms of positive and negative 'communication'. The darkening of insect cuticles is a process similar to the tanning of leather.

~~Some~~ We know rather little of human colour genetics, but we know a good deal of the activities of two genes which are incidentally colour genes.

Phenylketonuria is a recessive in which about 1 gm phenyl-pyruvic acid and 200 mgm. diphenylalanine are excreted daily in the urine (cf Harris 1953). Its most striking effect is to produce mental deficiency. We have no know why it ~~does so~~ affects the mind, but not the simpler functions of the central nervous system or those of most other organs we shall know something of the biochemistry of mental processes. It is certainly a metabolic rather than a renal effect. It is incidentally a colour gene, greatly lightening the hair colour.

Alcaptonuria is another recessive which causes the ~~skin~~ urine to

Reynolds, and Gr [illegible] Yeq) J.B.C. 184, .

[It is clearly of great interest, being the only enzyme known (to me at least) which breaks open a benzene ring.

excretion of about 4-5 gm./day of homogentisic acid, which rapidly forms a "melanin" on standing. It may possibly dilute the brain colour, and certainly leads to the formation of pigment in cartilage. This is visible in the ears and by hardening joint cartilages leads to a "rheumatic" condition. The changes in cartilage are clearly like those in insect cuticles. Rat's liver slices catalyse the formation of ~~4 fu~~ ~~homogentisic acid~~ 4-fumaryl-aceto-acetic acid from homogentisic acid. This then is then hydrolysed into fumaric and acetoacetic acids (Rawden and [illegible] 19.) (Fig). This enzyme may be absent or inhibited in alcaptonuria.

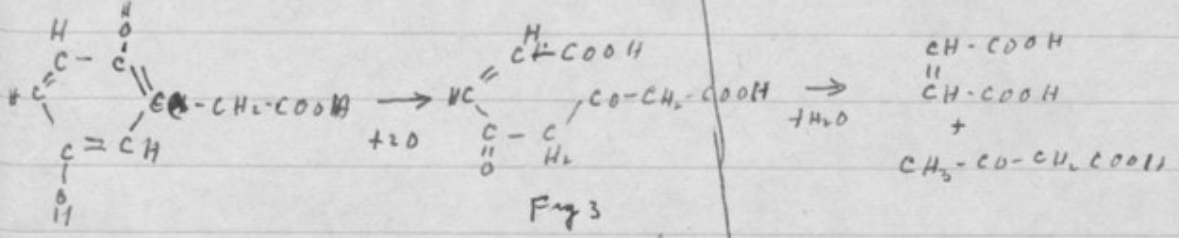


Fig 3

In a number of other mammalian abnormalities normal, or probably normal, metabolites are excreted in the urine. In any particular case one cannot say without further investigation whether this is due to a metabolic abnormality in other tissues or to a renal abnormality, presumably a failure to reabsorb from the glomerular filtrate. If the amount of the excreted substance in the blood is raised, like glucose in diabetes, it is reasonably sure that the kidney is not primarily responsible. However since renal function is influenced by hormones (notably the water output by pituitrin and the sodium output by one or more adrenal cortical hormones), it is always possible that another type of renal dysfunction, such as that in

Foster M. (1951) Journ. Exp. Zool 118, 211

That of yellows had a very weak activity. I've found "yellow" mice often from some black pigment. At pH 6.8

Dark pigment was formed.

~~In 1935~~ Foster (1951) ~~discovered~~ confirming the earlier work of Onslow, discovered that mouse skin contains a ~~tyr~~ true tyrosinase, and also a tryptophan oxidase. He worked with the skin of young mice, powdered under dry ice. The skin of albinos (cc) and the ~~white~~ unpigmented parts of the skin of piebald ($\frac{cs}{cs}$) mice had no tyrosinase activity. The skin of black mice had a powerful tyrosinase activity, specific for L-tyrosine. The activity was about doubled by 0·6 % iodoacetamide, and trebled by about ·005 M Ca Cl$_2$. It could be prevented by phenyl-thiourea. The uptake amounted to about 5 atoms O per molecule of tyrosine. Brown skin showed a lesser but still strong tyrosinase activity, and probably inhibited the black skin enzyme. Yellow skin certainly did so. On the other hand the white skin of piebalds seemed to be activated by black skin extracts. That of albino skin was not. Brown-agouty skin (grizzly brown hairs with yellow bands) took up O$_2$ after a latent period of ~~some~~ about 4 hours, or at once if copper or iodo-acetamide were added.

Yellow skin contained a powerful tryptophanase, also stereochemically specific. Stoicheometric data are not given, but yellow pigment was formed. Black and brown skins had a less effect, albino skin only after a latent period. This activity is not enhanced by copper, and somewhat depressed by iodo-acetamide. Unfortunately yellow pigmentation in mice is due to a dominant gene A^y, lethal when homozygous, and it cannot be assumed that the necessary yellows in the ~~rat or~~ guinea-pig or rabbit would behave in the same way. In fact one may guess that only yellow skins would not contain tyrosinase, but would not inhibit it unless they covered the gene. A grizzly yellow banded or black

Rothmann, Krylow, and Smiljanic (1940). Proc. Soc. Exp. Biol. Med 43, 208

hairs. Similarly it is ~~very unlikely~~ possible that Foster's albino mice carried the gene A, and that albinos not carrying it would have behaved differently. The use of iodo-acetamide was suggested by the discovery of Rothman, Krysa, and Smiljanic (1946) that human (European) skin contains an inhibitor of potato tyrosinase, the inhibition being removed by iodo-acetamide, and probably depending on sulphydryl groups.

Foster's paper clearly opens a new chapter in the subject. Further, since mammalian tyrosinase has not yet been obtained in solution, it offers the most hopeful method of fractionating it so that the different stages may be studied. The field is now wide open. Among the obvious questions (which Foster may well be answering as I write) are:-

Which skin powders if any, will act on tryptamine, ~~or~~ 3-hydroxy-tryptamine? or 3-hydroxy-anthranilic acid? Which, if any will catalyse the oxidation of 5-6-dihydroxyindole-2-carboxylic acid to the corresponding ortho-quinone, leucochrome. Can we catalyse the oxidation of tyrosine and tryptophan with mixtures of the skins of albino mice and the white parts of piebald mice, as we can produce full-coloured mice by crossing them? Are the inhibitors heat stable and dialysable? Can they be isolated? Would Crough B.A.L. turn a black mouse yellow?

However there are difficulties, which will later be discussed, in human genetics

Freedman, and Byers, (19). J.B.C. 1974,

the Fanconi syndrome, could be due to hormonal abnormality. Moreover if the renal tubule cells cannot absorb a particular substance, say phosphate, it is at least plausible that other cells may lack the same power. So a demonstration of abnormality in renal physiology is not a demonstration that other tissues are normal. A further difficulty, which Harris ( ) rightly stresses, is that we have generally no reason to assume that two indistinguishable human abnormalities, inherited, so far as we can see, in the same way, have the same genetic basis, and therefore the same biochemical disturbance. We cannot arrange for experimental matings between cystinurics of families A and B, though in this particular case he has made it highly probable that two different non-allelomorphic genes, one gene fully, the other nearly, recessive, cause cystinuria through interference with renal reabsorption. Whereas we can be sure that two cystinuric dogs are cystinuric on account of the same gene.

I therefore began with a case where the genetics and physiology are fairly clear. Most dogs excrete (and other mammals) excrete much more allantoin than uric acid. Normal Dalmatian dogs excrete about 0.2 % of their urinary nitrogen as urate and, Dalmatian coach dogs 2-3 %; nevertheless they excrete about twice as much allantoin as uric acid. Various authors failed to find a diminished content of urecase which oxidizes urate and to allantoin, in these organs. Friedman and Byers (19) found that in all dogs the allantoin clearance is equal to the creatinine clearance. That is to say they each excrete all the creatinine and allantoine in about a quarter of the plasma volume passing through these kidneys, which probably means that neither substance is reabsorbed from the glomerular filtrate. Normal dogs have a much lower urate and clearance,

which presumably means that they reabsorb this substance from the filtrate. In Dalmatians the uric acid clearance is the same as the creatinine clearance. ~~The normal~~ ~~gene~~ ~~enzyme~~ is absent in them thus ~~enzyme~~ of In consequence the ratio of uric acid to creatinine in the urine is heyher than normal, and about a third of their uric acid is excreted as such before it has had time to be oxidized to allantoin. Thus partial uricolysis in dogs is ~~compare~~ physiologically comparable to

(continued ~~in Vol 2~~)

renal glycosuria in man. The partial uricolysis (lack of allantoin) in the pe Pomatis is due to lack of uricase. Finally as we shall see the hyperuricaemia of gouty man is due to a rise in the renal threshold for uric acid or at least a sluggishness in secreting it, the opposite of the a reduction in the opposite direction to that found in the Dalmatian dogs.

(go on to Vol 2)

r rear a batch of larvae on a defined diet, it is necessary that the bottles in which they are bred and the flies put into them should be completely sterile. ~~They constitute~~ The larvae burrow into the food and cannot be transferred to fresh food like rats. At the concluding ~~end~~ of the experiment each bottle must be checked for mycological and bacterio-logical sterility at the end of a week or more.

(auxotrophism)

It is surprising that even one case of a nutritional need of the Neurospora type is known in insects. However the method by which it was discovered may yet enable the discovery of numerous similar cases. The inversion In(2LR)40D in Drosophila melanogaster, in which the central portion of the third chromosome is inverted relative to the ends, ~~has~~ is most readily detected either by microscopical examination of the chromosomes or by its interference with normal crossing over. It also produces somatic effects which depend on the state of the culture medium. The most conspicuous is disarrangement of the eye facets. These are "cured" if the larvae are grown in a medium containing plenty of hydrolysed nucleic acid. Normal Drosophila melanogaster grows on a medium containing 5% sucrose, 13 aminoacids, cholesterol, lecithin, and vitamins. The addition of nucleic acid speeds up growth and raises the survival from 63% to 94%. On the other hand In(2LR)40D dies on the synthetic medium, while 93% survive when nucleic acid is added. It was found that adenine, or adenine nucleoside or nucleotide were equally effective, whilst guanine ~~used as~~ allowed a few individuals to develop. These flies are therefore "adenine-less", presumably because two loci concerned in adenine synthesis have been separated. Similar reactions to diet are not at all uncommon with Drosophila structural mutants, though a few show the opposite behaviour, abnormalities being more marked if large amounts of yeast are added to the diet. But ~~Just because~~ geneticists prefer to work with mutants whose manifestation does not depend on trace constituents in the diet. This is not a mere symptom of laziness. It is much easier to feed rats than Drosophila larvae on a diet deficient of a given vitamin or other constituent. This is because the normal diet contains live yeast, and to

Lwoff A. (19)

Lwoff A. (1949). Unities biologiques . . . . . 7.

Chap X.7.

Extranuclear influences on biochemical activity, including training.

Once we depart from the study of characters determined by genes we are faced by considerable difficulties. Of these the difficulty or impossibility of distinguishing between the transmission of characters determined by self-reproducing extranuclear structures and by viruses. Another is the problem of training in unicellular organisms, which I have deliberately postponed.

The absence or abnormality of chloroplasts in higher plants may be due to nuclear genes. Or all the descendants of abnormal chloroplasts may be abnormal. In this case the inheritance is usually maternal, since it is unusual, though not unknown, for chloroplasts to be transmitted through pollen tubes. In flagellates the chloroplasts are often countable, and different species may display every gradation of behaviour from one satisfying the theoretical views of Weissman, to one satisfying those of Lamarck. (for references v. Lwoff 19, 1944)

Haematococcus pluvialis remains green indefinitely.

Euglena gracilis loses chlorophyll after a few weeks in darkness, but retains 8 to 10 plastids. Even after 15 years in darkness it becomes green after a few hours in light. This is like the 'training' of bacteria and yeasts.

Euglena mesnili normally possesses about 100 chloroplasts. In darkness they remain green but their number falls off, and may fall to 10 or 12 after one months. Even 10 individuals without plastids may be formed at a relatively division. Unlike plastidless individuals of Rhizobium

In connexion with the comparative autonomy of chloroplasts in higher plants, Metzner's (1952) finding that they contain DNA as well as RNA may not be irrelevant.

Metzner H. (1952). Biol. Zent. 71, 254.

Ephrussi B. (1944). Unités biologiques douées de continuité génétique, 165, and in a forthcoming book.

and, though they can ferment glucose, cannot oxidise it.

Etherfeli ~~which single large plastid occasionally fails to divide, in which~~ which are formed when its single large plastid fails to divide, plastidless E. mesnili can reproduce, though they grow slowly, and have never been kept over many generations. Here then is a perfect Lamarckian example of irreversible hereditable loss of a function through disuse. It is apparently vain to hope that the existence of such a series of organisms will prevent dogmatic assertions ~~on this topic~~ both as to the non existence of this phenomenon and of its universality.

Lic

E. pbrussii (1949 and in the press)

investigated a similar case in yeast. Normal yeasts occasionally produce small slowly growing cells, which do not oxidize "nadi" (α-naphthol + p-phenylene diamine) to a characteristic blue color, as the normal yeast cells do. These cells segregate by "mutation", one of a pair being small in about 0.4 % of divisions. If acriflavine is added to a culture, ~~mutation~~ there is no effect unless cells divide, but mutation occurs at most divisions, and after 24 hours less than 1% of the cells are normal. The mutation is irreversible. The mutants lack cytochrome oxidase and succinic dehydrogenase. When a culture of the small form is grown in a normal medium and crossed with the normal, all the spores in an ascus usually give normals, whereas other characters (e.g. "adenineless" producing a red pigment) usually give 1:1 segregation. However about 1% of tetraspores give small cells. E. pbrussii showed conclusively that this was not due to Mendelian segregation of several genes. He later obtained a mutant in which a similar obligatory amitochondriosis was due to a single gene.

Quite recently Mitchell and Mitchell (1952) have obtained the first cytoplasmically determined mutant in Neurospora crassa. This is "poky", a slow grower for reasons at present unknown.

Mitchell M.B. and Mitchell H.K. (1952). PNAS 38, 442.

von Wettstein W.J. (1948) J.B.C. 173, 691

called Huppa.

A single cell may contain about 1000 of these particles

If the normal yeast cell has about 1 cyto self-reproducing cytoplasmic particles containing the respiratory enzymes which are distributed at random, we should expect to get about 1 cell in 512 without them in binary divisions, and about 1 in 5 in quaternary divisions. If acriflavine prevents the particles from dividing the observed results can be explained.

In Paramecium aurelia some clones produce ~~soluble substances of unknown composition which kill other members of the species. This "killer"~~ character is due to cytoplasmic particles called "kappa" paramecins which kill other members of the same species. Van Wagtendonk (1948) showed that they are deoxyribonucleoproteins. They are spontaneously inactivated at all pH's, though most slowly at P.C. The inactivation is accelerated by several proteinases and by deoxyribonuclease but is probably not ~~at~~ in presence of Mg^{++} or Mn^{++}. Paramecin is produced by (and may be identical with) Feulgen staining particles in the cytoplasm of the killers. These will only multiply in presence of a nuclear gene K. (cf Sonneborn 1943), But though they may persist for at least 6 cell divisions after K has been lost. If animals are kept in a medium where fission is retarded, they may divide more rapidly than the Kappa particles, and finally the large majority cease to be killers. Clearly kappa may be regarded as a kind of ~~subcellular~~ virus, but of sort is peculiar in protecting its hosts against effects which are fatal to "uninfected" individuals. On the other hand any mutation with this peculiar property is clearly favoured by natural selection.

A number of self-propagating cytoplasmic properties are known in

Darlington C. D. (1944). Limites biologiques données de continuité génétique. 123, Paris C.N.R.S.

Baltzer J. J. (1934) Am. J. Clin Path 4, 430

"Scrapie" in sheep is a virus disease transmissible by inoculation. It can also be transmitted by a "scrapie ram" who does not himself show it, to his lambs, though not necessarily to their mothers (Andrewes 19). Such a method of transmission ~~of cancer~~, which would simulate an "irregular dominant" is not excluded in the case of cancer.

Rettrian, R. J, Dempster E., and Turner H (1944). J B C 154, 491
Rettrian R. J. (1951). Genetics. 36, 54

They therefore compared rats of a rapidly growing inbred strain F, and a slower growing strain J.

higher plants. ~~Some can be Attempt~~ Some are transmitted purely maternally. Of these again some can be transmitted by grafting. Darlington (1944) and Lysenko (1949) have reviewed ~~these matters~~ this topic, but it does not seem to me that the evidence is at present sufficient to justify the wrecking claims made by either of these authors, though ~~this may not always~~ such evidence may be obtained.

In mammals several characters are transmissible by milk. Mammary cancer in mice is due to a virus which is regularly transmitted to the offspring or foster-children through the milk, and (Bittner 1937 and later) ~~apparently from male to female mice to their mates~~ (mammary cancer). However two points are to be noted. In the first place the virus causes acinar hypertrophy during the first lactation or earlier, while cancer may not develop till much later. Secondly the age at which cancer develops depends on genes transmitted in the ordinary way. The virus is thus not harmful to all mice, and we must be prepared to find harmless characters transmitted in a similar manner.

A case which will doubtless be further investigated was described by Rahman, Dempster, and Tarver (1942) and Rahman (1951). Growth rates in animals are ~~gained~~ in part genetically determined. They may depend in part on the rate at which amino-acids are incorporated into tissue protein. The authors incubated liver slices with 1·33 m M DL-methionine containing radio-sulphur, and isolated methionine sulphur from the protein hydrolysate after 2 hours' incubation. Rats of Strain F gave about 0·4 % replacement, strain J about 0·2 %. Most remarkably however two rats of

The babies were transferred after they had absorbed at least some colostrum from their mothers, and probably too late to ~~absorb~~ obtain any from their foster mothers.

Shamyan V.A., and Yudin, V. M. (1949). The situation in biological science, pp. 250 and 405.

Strain J when suckled by F mothers not only grew quicker, but were (indeed quicker than either J or F nursed by their own mothers) but incorporated methionine more rapidly than F suckled by F. F suckled by J showed no increased growth, but incorporated methionine rather quicker than when suckled by F. The hybrids, regardless of what between the two strains at first resembled their mothers in methionine replacement, but when adult resembled strain F. The back cross to strain J showed considerable evidence of segregation for methionine synthesis. So far as I can interpret the statements of Shaumyan and Yeskin (1944) it would seem that Soviet workers have found considerable maternal effects on economically important characters in cattle and sheep. Unfortunately their original data are not available to me, nor do I know whether they have compared the effects of milk with those of prenatal environment. This would seem to be an obvious slip in applying the theories of Mitchurin to animals, even though Rutman's back cross Rutman's data give no reason to suppose that in this case characters induced by milk are handed on indefinitely. It will be of great interest to determine the nature of the substance in milk responsible for this effect. As the stocks differ in growth rate, it is likely to have a general effect on protein synthesis rather than a specific one on the incorporation of methionine.

250

I now pass to a consideration of the effects of "training" and similar processes in unicellular organisms. When a culture of bacteria is placed in a new medium, some kind of adaptation is often found after a number of cell generations. This may be due to training, to selection of mutants, or to both. The simplest way to demonstrate training is to keep the organisms in a medium in which they will not multiply. As can

go on to Vol 2

Monod J. (1944). Unités biologiques douées de continuité génétique, 181.

" " (1950). B cochem Soc Symp. 4, 51

a can "learn" to utilize maltose, some can "learn" to utilize lactose. After adaptation to maltose they

It is not for example an α-glucosidase; so

example of work on training which, ~~from a genetical point of view,~~ both from the genetical and biochemical points of view, gives extremely clear results I take that of Monod (1949 a, b.) on Escherichia coli. He worked on the strain ML. ~~(no showing evidence of sexual reproduction (cf p. ——)~~. Some cultures of this strain ~~although can utilize maltose~~. They contain an amylomaltase, a new type of enzyme, which reversibly converts half the glucose residues of the maltose into starch (polysaccharide reacting with iodine).

n maltose + 4-α-Glucosido-glucose $\rightleftarrows$ n glucose + (glucose)$_n$

cf. n glucose-1-phosphate $\rightleftarrows$ n phosphate + (glucose)$_n$.

~~Again, though none of these bacteria can overdye lactate at an appreciable~~ ratio after having grown on glucose, they "learn" to do so, the training ~~being~~ complete in about 3 hours at 37° C. This learning capacity is absent in ~~starks~~. So far this enzyme has proved to be quite specific to lactose, not maltose, as has the corresponding lactase to lactose. ~~The capacity~~ to form ~~these two enzymes depends~~ The enzymes can be extracted from adapted bacteria and partially purified. None have been found in extracts of unadapted bacteria, nor have attempts to activate them succeeded. The capacity to make these enzymes depends on two different genes. Monod has obtained stocks of the four different possible types M^+L^+, M^-L^+, M^+L^-, M^-L^-, and observed mutations from one to the other, spontaneous or provoked by ultra-violet radiation. ~~One of these genes exists in Lederbergs~~ ~~and K12 strain, and shows no real linkage relations with other genes.~~ As ML is unusual the proof of the genic nature of the determination is incomplete. But

showing linkage with others, and

formation of lactase

in a few hours. There is thus in this case no evidence that ~~any~~ more of the enzyme is made after it ceases to be ~~in a few hours~~ used.

~~and to~~ Once the lactase is developed the sugars formed from starch oxidized.

L. Bacterium can oxidize galactose, but it does not induce them to form lactase.

and even more interesting if it were a nucleoprotein.

Lederberg's (cf p.) sexual strain K12 has a gene pair controlling the presence or absence of capacity to form lactase in presence of lactose. This is presumably not the same as Monod's L^+ gene, as the enzyme is an unspecific or relatively unspecific β-galactosidase. But it is conceivable that homologous genes, in different organisms, may control the syntheses of enzymes with a different range of specificity

Adaptation is fairly complete in 4 hours when washed cells are suspended in phosphate buffer, lactose, and ammonium sulphate. It is also fully reversible. Adaptation occurs not only in presence of lactose but of galactose, although there is no reason to think that the enzyme acts synthetically! On the other hand amylomaltase is not formed in presence of glucose and starch, although it can form maltose from them! Adaptation only occurs partially in the absence of a nitrogen source, and is inhibited by 2-4-dinitrophenol, $NaNO_2$, and phage infection, all of which inhibit protein synthesis. In yeast (19) found that hydrolysed ribonucleic acid, but not desoxyribose nucleic acid, accelerate adaptation, as might be expected from the part which ribonucleic acid plays in protein synthesis. So far as I know no adaptive enzyme has been crystallized. It would be of great interest to [illegible] discover whether, say, an adaptive lactase contains a galactose residue.

Neurospora forms several adaptive enzymes. In some cases it appears that their synthesis requires the presence of more than one normal gene. The adaptation is completely lost not only on sexual reproduction, but on asexual reproduction by microconidia which contain a nucleus but

The same is true in Aspergillus nidulans. ~~Thus~~ All the nicotinic acid-requiring mutants in this species are adaptable, growing at an almost normal rate in the absence of this substance after a lag of several days. This adaptation is not transmitted through the conidia.

Wynge, O. and Roberts C. (1948). C.R. Lab. Carlsberg, Ser. Physiol 263

Lindegren (1945) Amer. Mus Bot. Gard 32, 104

Spiegelman S, Lindegren C.C., and Lindegren G. (1945). PNAS. 31, 95

Spiegelman S (1946). Cold. Spring Harbor Symp. Quant. Biol. 11, ~~245~~ 256.

little cytoplasm.

Some lights I suggest the situation is far from clear. Winge and Roberts (1948) showed that ~~yeasts could~~ different yeasts could differ regarding their fermentative capacities in the following way. Saccharomyces C. vordae ferments maltose without adaptation, and ~~reacts to~~ galactose after adaptation, reaching half the maximum rate in about a day. Saccharomyces chevalieri cannot adapt to maltose, and adapts slowly to galactose, reaching half the maximum rate in about 8 days. On crossing the species the asci give rise to a variety of haploid forms. Some of the segregants ferment maltose at once, others after rapid adaptation, others after slow adaptation, others not at all. Three genes appear to be concerned. The difference as regards ~~galactose~~ adaptation to galactose depends on a single gene

Lindegren (1945) and Spiegelman, Lindegren and Lindegren (1945) [cf. also Spiegelman 1946] crossed Saccharomyces Earlsbergensis, which can ferment melibiose (6.α galactopyranosido-glucopyranose) after adaptation, with S. cerevisiae, which cannot. The initial results were not clear, but however suggested that several genes were segregating, ~~but~~ further back-crosses gave single gene segregation. If crossing ~~and~~ segregation, and the growth of a new haploid generation took place on a melibiose-free medium, ~~half~~ the progeny of half the spores could learn to ferment melibiose, that of the other half could not. If however conjugation, segregation, and further growth took place in presence of melibiose, all the progeny could ferment it, even after over a 1000 generations. When these cultures were kept in the absence of melibiose or a nitrogen source, so that hardly any vegetative growth occurred, they lost

The word "plasmagene" has also been used. I should personally like to revive Contagnes (1902) word "mnemon" for such hypothetical structures, keeping the word plasmagene for stabler components.

(Lindegren 1949)

Lindegren C. C. (1949). The yeast cell, its genetics and cytology. St Louis.

them capacity to ~~no~~ ferment melibiose in three weeks at most. Half could regain it on further training, half could not. It would seem then that the gene for adaptability to melibiose produced something in the presence of melibiose which can reproduce itself in this even after the gene has been lost by segregation, but which needs melibiose to reproduce itself. This something could be the enzyme, or a "cytogene" producing the enzyme. There is no question of a store of enzyme being gradually used up. Such a store to last 100 generations, would have to consist of at least 2^{1000} molecules. The earth only contains about under to 2^{170} atoms. Unfortunately it has been impossible to repeat this remarkable experiment. It must be remembered that a named species of yeast may be very heterogeneous, and that a yeast stock, even if kept meticulously from contamination, is under very heavy natural selection and must be expected to change in the course of some years. As Catchside points out, there are undoubted cases in higher plants where genes sometimes produce e.g. an alteration in extranuclear components which perpetuates itself in the absence of the initiating gene. These are not however adaptive. But Lindegren and Spiegelman's claims are in no way contrary to the generally accepted principles of genetics (or Mendel-Morganism)

It is perhaps worth emphasizing that for an adequate understanding of these phenomena "forgetting" is quite as important as training. It is not particularly surprising that a cell if under a novel chemical stimulus should make a hundred or even 100 or even several thousand molecules of an enzyme where none existed before, and that one or more genes should be needed for such a synthesis. The stem of a Himalayan rabbit makes melanin if and only if it is kept sufficiently cold, and crosses of albino

Hinshelwood C (1953). Sympos. Soc Exp Biol. (in press)

Ryan F.J. and Lederberg J. PNAS. 36, 163

Ryan F. J. Cold Spr. Harb. Symp. Quant Biol 11, 215.

Sherman T. C., and Ryan F.J. Genetics 33, 221.

examples analogies could be given. Now is it surprising that when such an adapted cell divides, even in the absence of the stimulus such as an abnormal sugar, about half these enzyme molecules should go to each daughter cell. But a bacillus with a dry weight of 10^{-13} gm. could only contain a million enzyme molecules of molecular weight 60,000, and certainly contains much fewer. Of any particular kind, probably less than 10,000 If new ones were not made, these after about 13 generations most of the progeny would not possess even one molecule. Now "forgetting" or whatever word is used for the loss of training can occur in a dozen or so generations, or even without cell division at all. But a learned capacity can be inherited for several hundred or 6300 generations in the absence of stimulus (Hinshelwood 1953) or over 1000 in the absence of a gene needed for its induction.

There is thus no question that sometimes (but not always) the adaptation involves not merely the production of catalysts after under the influence of a new stimulus, but their continued production after it has ceased. In some sense then it must be regarded as a ~~biological~~ hereditary character in the biological sense, and not not as a persons transfer of material.

Before our final discussion we must consider a further point. Adaptation can occur as the result of gene mutation, and in asexual organisms it may be very hard to distinguish this from training, though Hinshelwood has, I think, succeeded in doing so in some cases. Where crossing and genetical analysis are possible, the distinction is much easier, and the question then arises as to whether the adaptive mutation was induced by the change in the medium. The most complete answer to these questions has probably been given by Ryan and Lederberg (1946), Ryan (1947) and ~~Ryan and~~ Stanley and Ryan (1948)

At lower lysozyme concentrations back mutation was still more frequent. To estimate lysozyme by this method

Hinshelwood, C.N. The (1946). The chemical kinetics of the bacterial cell (Oxford)

A leucineless mutant of Neurospora crassa, l_1, usually grows at a standardized rate on media containing small amounts of leucine. Thus on a medium containing 10 mg./litre leucine, seven cultures produced 17.2-18.0 mg of mycelium on 5-0 ml. Three others produced 19.0, 20.1, and 44.8 mg. Genetical analysis by back-crossing to a different l_1 strain showed that back-mutation had occurred. ~~I got adequate results~~ it is better to use a double mutant $l_1 l_2$. Double back mutations are extremely rare. It was further found that the frequency of back-mutation was much greater ~~in cultures~~ on media containing ~~only~~ little leucine, as if the mutation were adaptive. Now the mutants start as heterokaryons. Artificial heterokaryons were made up each in which the two types of nuclei differed in respect of other genes as well as l_1 and it was shown that l_1 nuclei multiply quicker than the wild type in presence of moderate amounts of leucine such as 15 mg/litre. ~~The cause of the competition is not clear, but the~~ This is not so on the minimal medium. The reason for the successful competition is not clear. But it accounts for the appearance of adaptive mutation. In other cases, for example resistance to sulphonamide, both cytoplasmic adaptation and mutation have occurred in Neurospora.

Q We can now consider the standpoint adopted by Hinshelwood, who has worked on adaptive changes in bacteria, and to a less extent, in yeasts. This is clearly stated in his book "The Chemical Kinetics of the bacterial cell" (1946) (p. 10)

He believes that the growth is to be explained by autosynthesis of enzymes He gives some examples of inorganic reactions where, to quote his own words (p. 16)

"catalyst + substrate = more catalyst + product."

and continues". Moreover in a constant medium the various enzymes

(continued in Vol. 2)

f-98b

D.O.No. 1037/BG

From

Prof. P.N.Mehra,D.Sc.,F.N.A.Sc.,
Professor & Head of the Botany
Department, Panjab University,
Khalsa College, Amritsar.

Dated 29th December,1951.

Dear Prof. Haldane,

I would very much have liked to meet you at Calcutta but for reasons of health I am unable to do so. On behalf of the Vice-Chancellor of the Panjab University I extend to you an invitation to visit our institute at Amritsar. We will make the necessary arrangements for your stay here. I shall be greatly delighted if you can accept this invitation.

Yours Sincerely,

P.N. Mehra

University Professor of Botany.

Prof. J.B.S.Haldane,M.A.,F.B.S.,
C/O The General Secretary,
Indian Science Congress Association,
1, Park Street,
Calcutta.

Mangelsdorf P & Fraps G. Scient 73, 241, 1931

Y genes	B.-carotene x 10⁶	
3	4.50	and reasonable
2	3.00	
1	1.35	
0	0.03	

West F.W., Loden A.L. & Zechmeister L
Plant Physiol 14, 41, 1942

Tomato

	Skin	Flesh	Lycop	Xanth.	β-car
YR	yellow	red	20.2	10.3	4.9
			21.9	6.5	2.0
yR	white	red	0	2.9	1.5
Yr	yellow	yellow	0	6.3	1.8
yr	white	yellow			

(*Reprinted from Nature, Vol.* 169, *p.* 513, *March* 29, 1952)

FIFTY YEARS OF GENETICS

Genetics in the 20th Century

Essays on the Progress of Genetics during its First 50 Years. Edited for the Genetics Society of America by Prof. L. C. Dunn. Pp. xiii+634. (New York: The Macmillan Company; London: Macmillan and Co., Ltd., 1951.) 37*s*. 6*d*. net.

THE Genetics Society of America is to be congratulated for having organized in September 1950 a meeting to celebrate the fiftieth anniversary of the re-discovery of Mendel's principles. This volume brings together twenty-six essays presented by distinguished American and European geneticists. They survey the developments and the outlook of genetics, its relations to biology in general and to specific fields in particular, and some of its applications to agriculture and medicine. In their gratifying variety of styles, subjects and objectives, none is irrelevant and one, by T. M. Sonneborn, may mark a turning point. In a short review it is impossible even to mention every one of these essays, let alone to do them justice.

R. B. Goldschmidt's opening address is a cheerful, almost enthusiastic, review of the impact of genetics on science; it comes as a surprise from the man who has been persistently ahead of the times with constructive criticism. It is to be hoped that this is only a natural effect of the elated atmosphere of the jubilee celebrations and not a reaction to the attacks now being waged against genetics. Three essays of historical character follow, one by H. Iltis on Mendel's life; one by Conway Zirkle on the knowledge of heredity before 1900; and one by W. E. Castle on his recollections of the first ten years of Mendelism in America. The last brings home the very important part played by mammalian genetics before the appearance of *Drosophila*. One episode is worth reporting: as late as 1909 Morgan was attacking the chromosome theory of inheritance; yet only three years later he and his unique team produced the theory of the gene.

The essay by H. J. Muller expounds the theme of the "gene as the basis of life", which he first outlined in 1926. The value of this idea has gained momentum in the past ten years during which the studies of heredity, embryology, immunology and biochemistry have come to share interests and techniques. It is an idea of great unifying value since it makes heredity,

differentiation and evolution—life, as we know it, in one word—inevitable if one assumes a primitive structure capable of promoting the synthesis of more of its own kind, and of mutating, that is, of changing and promoting the synthesis of the changed kind. These are the properties rightly or wrongly attributed to the genes, the viruses and other 'self-duplicating' particles of to-day.

As the recent work on phage suggests, however, 'self-duplicating' particles, as we find them to-day, are meaningless without the highly organized synthetic mechanisms of the cell, of which mechanisms they are part and parcel and in which they act as specific primers. Sonneborn's essay—and one by B. Ephrussi—deal precisely with this central question of the interactions between genes, non-nuclear 'self-duplicating' structures, other cell systems structurally organized or not, and the environment. With unprecedented clarity these two essays state the nature of the problems for unifying genetics and development.

Another most valuable theoretical essay, on biometrical genetics, is by K. Mather. Genetics has learned to walk before trying to run : there is little doubt that its success has been due to the fact that the study of discontinuous variation has been dealt with first. Continuous variation, neglected since 1900, is just coming into its own again : its biological basis is now known to be the same as that of discontinuous variation ; but Mendelian analysis cannot be applied to it. New techniques have to be invented. Mather's essay sets out lucidly the kind of problems involved and how they are beginning to be attacked. Perhaps as the statistical work of R. A. Fisher, Sewall Wright, J. B. S. Haldane and S. S. Chetverikov about 1930 reconciled Darwin and Mendel, this new biometry, in which Mather, J. L. Lush and M. Lerner lead, will ultimately reconcile Galton and Mendel.

Three essays on chemical aspects of genetics are by G. W. Beadle, by T. Caspersson and J. Schultz, and by A. E. Mirsky. An excellent summary of the genetics of antigens in man and animals is given by M. R. Irwin. J. Lederberg, who is responsible for one of the most spectacular recent advances in genetics, has contributed an essay on the genetics of bacteria. It is to be regretted that there are so many non-essential technicalities in it that the non-specialized reader may lose sight of the beauty of Lederberg's fundamental work.

Of the many other essays, I may mention a clear summary of the 'hybrid corn' developments by P. C. Mangelsdorf ; a discussion on the evolution of

cytogenetic mechanisms by M. White; one on population genetics by T. Dobzhansky; a short but stimulating one on human genetics by L. S. Penrose; and others by C. D. Darlington, A. H. Sturtevant, C. C. Little, J. L. Lush, A. Muntzing, L. H. Snyder and J. C. Walker, on subjects ranging from the meaning of Mendel's determinants to the genetics of resistance to diseases, from the genetics of cancer to animal and plant breeding.

In the closing essay by Julian Huxley, written in his lucid style, the part dealing with the novel mechanism of evolution emerging from the development of mind and of transmission by tradition is most stimulating.

Clearly this volume is one that no thoughtful biologist can ignore. G. PONTECORVO

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Production of Heterozygous Diploids in Filamentous Fungi

In most filamentous fungi the nuclei are haploid throughout the life cycle except for the zygote nucleus which is usually diploid and which immediately undergoes meiosis. Undoubtedly, as an accident of nuclear division, polyploid nuclei (that is, diploid or higher in the hyphae, tetraploid or higher in the zygote) may occur as they do in other organisms. Indeed, the artificial production of polyploids has been claimed[1]. In none of these cases is there conclusive evidence of polyploidy, though in one case[2] there is some. If polyploid nuclei arise, or can be induced to arise, as rare accidents in division, the problem is how to recognise the hyphae carrying them and how to make sure that these nuclei are polyploid. These conditions have been fulfilled in the development of a technique for the production of polyploids in the homothallic *Aspergillus nidulans*. This technique has now been applied with consistent results to the production of polyploids heterozygous for known genetic markers; it can be applied, undoubtedly, to any other filamentous fungus in which heterokaryosis occurs and in which there are uninucleate vegetative cells at some stage in the life cycle.

The technique is based on the following reasoning. When heterokaryotic cells are formed between two strains, differing in two nutritional requirements and/or two morphological characters, the two types of nuclei are segregated into individual uninucleate cells (for instance the conidia in *Aspergillus nidulans*). Following

[1] R. Bauch, Naturwissenschaften *29*, 503 (1941).—E. R. Sansome, Nature *157*, 843 (1946).—E. S. Beneke and G. P. Wilson, Mycologia *42*, 519 (1950).

[2] E. R. Sansome, Trans. Brit. mycol. Soc. *32*, 305 (1949).

plating, these conidia give origin to colonies which are of either one or the other parental type. If, however, a conidium has been formed which carries a diploid nucleus with one chromosome complement from each parent strain, a colony will arise which differs in characters from both parent strains. In most cases one can reasonably guess in which way the diploid heterozygote should differ from the parents. For instance, if one parent requires one growth factor and the other parent a different growth factor and each of these requirements is known to be genetically conditioned the diploid will probably require neither growth factor. Similarly if one parent differs from normal (green) in having yellow conidia and the other in having white conidia and the two differences are known to be determined by mutation in two different autonomous genes, the diploid (which is heterozygous for both) will probably have green conidia. The reasonable assumption in both cases is that the mutant characters—requirement versus non-requirement mutant colour versus normal colour—are recessive. The diploid will, therefore, be distinguishable from the parental types; it may also be selected out of the mass of parental types by using non-supplemented media.

An example, out of many, in which both colour of conidia and nutritional requirements were used as markers will illustrate the technique in operation. A heterokaryon between a strain requiring lysine and having yellow conidia and one requiring adenine and having white conidia was treated for 5 hours at 37° with d-camphor vapour. Conidia developed after treatment were plated on a medium lacking adenine and lysine. Out of several hundred thousand conidia only a few colonies grew and these had green conidia and, obviously, did not require either growth factor. A variant of this technique is that of treating with camphor vapour a heterokaryotic colony and letting it grow after treatment: in this case the diploid may arise as sectors of green colour in the part of the colony developed after treatment.

That the strains obtained by this technique actually carry diploid heterozygous nuclei is shown by:

(1) the phenotype of the strains;

(2) analysis of the ascospores, among which the expected recombinant types are found;

(3) the fact that all diploid heterozygotes so far produced undergo rare somatic recombination resulting in further diploids homozygous for one or more of the "markers" and still segregating for the others[1];

(4) the diameter of the conidia which is approximately 1·3 times that of the parent (haploid) strains;

(5) verification of the number of chromosomes at meiosis[2];

(6) the fact that the diploid strains, though tending to become homozygous for any markers for which they were originally heterozygous, remain diploid on indefinite subculture by means of conidia or hyphae, but not always on subculture by means of ascospores.

Haploid strains of *Aspergillus nidulans* invariably produce eight spored asci. The above mentioned diploids show in their young perithecia a large number of zygotes in meiosis which, however, result in few mature asci. These asci, unexpectedly, have mainly sixteen spores. The extremely low viability of the ascospores from diploids has not made possible a quantitative genetical analysis of tetraploid inheritance, but it has shown that both haploid and polyploid ascospores are formed.

J. A. Roper

Department of Genetics, University of Glasgow, September 5, 1951.

Résumé

En exposant aux vapeurs de camphre un mycélium de l'*Aspergillus nidulans* hétérocaryotique pour deux types de noyaux génétiquement marqués, on a obtenu des noyaux diploïdes hétérozygotes. Il est probable que ces noyaux diploïdes sont produits par l'inclusion dans un seul noyau de deux groupes haploïdes de chromosomes-fils résultant de la division de deux noyaux de types différents. On a réalisé ainsi une sorte de caryogamie artificielle. Des souches diploïdes sont obtenues par l'isolement au micromanipulateur de conidies uninuclées diploïdes. On peut vérifier le fait que les souches sont diploïdes en observant

[1] G. Pontecorvo and J. A. Roper, J. gen. Microbiol. (in the press).

[2] G. Pontecorvo (unpublished).

1° leur phénotypes;
2° le nombre de chromosomes;
3° la ségrégation et la recombinaison des gènes dans les ascospores;
4° la recombinaison somatique;
5° le diamètre des conidies.
