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A number of years ago it was shown by Schiff, Lussana, and others that the liver has the power of arresting certain poisons on their way from the intestines, and thus preventing them from reaching the general circulation. In consequence of this a dose of these poisons which would have proved fatal if injected directly into the general circulation had no effect when injected into the intestine or into the portal vein, provided always that the injection was made sufficiently slowly to allow the liver to act upon the poison. The action of the liver in thus preventing the toxic effects of poisons appear to be of a twofold nature—(1) It arrests the poison in its passage through the portal circulation, and excretes it into the bile, whence it passes again into the intestine. This action is exerted upon mineral poisons and possibly upon some organic poisons. (2) It actually destroys the poisons, converting them into substances which are either non-poisonous or only feebly toxic in comparison with the original poison. This action appeared to be exerted upon various organic poisons. (3) In destroying organic poisons the liver appears in some cases not only to remove their lethal power, but also to convert them into antidotes to the original poisons. Thus it was shown by Fraser that when increasing doses of snake venom were injected into an animal a certain amount of toleration was established, so that finally fifty times the dose which would at first have been fatal could be injected into the animal without injury. In such animals Fraser found that not only did the serum of the animal contain an antivenin, but that the bile also contained it, so that both the serum and bile of the animal thus immunised had an antidotal power when injected along with a dose of venom into an animal which had not been immunised.

Six years ago, while preparing an address for the International

Note.—The diphtheria toxin used was of such a strength that when 0·1 c.c. was injected into a guinea-pig weighing 500 grms. it caused death in forty-eight hours.

The toxin was kept at a constant temperature of 37° C. during the whole time of the artificial circulation by being passed through a spirally wound tube immersed in a water jacket fitted with a thermometer and thermo-regulator, and heated by a ring-shaped Bunsen burner.

Congress at Moscow, one of us visited the Pasteur establishment for preparing antidiphtheritic serum in Paris, and was much struck by the observation which the director made that not only was there a considerable difference of protective power between sera obtained from different horses, but also that it varied in the serum of the same horse obtained at different times. Fraser's experiments on venin suggested the possibility that part of the antitoxin formed by the horse might be removed from its blood by the liver, and, being excreted by the bile, might be eliminated in the fæces, so that an attack of diarrhœa in a horse might possibly lessen the antitoxic power of the serum. On inquiry of the director, he found that no observations had been made with regard to this point; and so far as we know no experiments have been made upon the subject.

The following research was undertaken with the view of ascertaining—

1. Whether the liver has the power of destroying or lessening the lethal activity of a toxin in the same way as it diminishes the poisonous power of vegetable alkaloids such as morphine, strychnine, veratrine, and quinine.

2. Whether it forms from a toxin an antitoxin which is excreted by the bile.

It will be seen from the experiments which we describe that the liver appears to have the power of diminishing the lethal action of the diphtheria toxin which is circulated through it. This power is exerted upon the toxin whether it be circulated alone or mixed with blood. The bile appears to have a certain antitoxic power, for when 1 c.c. of diphtheria toxin had been mixed with the same quantity of bile obtained after circulating toxin through the liver, it only killed a guinea-pig weighing 250 grms. on the fourth day, whereas one-tenth of this same toxin unmixed with bile killed a guinea-pig of double the weight in half the time. In order to ascertain if possible what constituent of the liver had the power of lessening the activity of the diphtheria toxin, the expressed juice of a liver through which toxin had been circulated was inoculated along with diphtheria toxin. This showed a certain antitoxic power. From such expressed juice a nucleoproteid was separated which, when mixed with diphtheria toxin, exerted a marked antitoxic action distinctly stronger than that of the liver juice.

We append some details of the experiment upon which the foregoing remarks are based:—

A.—Circulation of Diphtheria Toxin through the Isolated Liver of a Rabbit.

The conditions of experiment were as follow:—

The abdomen having been laid open by a crucial incision, all the blood vessels, both hepatic and portal, were exposed and tied. Cannulæ were placed

in the duodenal branch of the portal and in the suprahepatic veins. The portal cannula was connected with an apparatus so arranged as to give an intermittent flow of fluid kept at a temperature of 37° C., at a pressure of from 10 to 12 in. of the circulated fluid. The organ was first freed from blood by circulation of normal saline solution. The saline solution was then replaced by diphtheria toxin, 100 c.c. of which introduced by the portal cannula was allowed slowly to escape by the cannula in the suprahepatic vein. During the process of artificial circulation the liver was allowed to become moderately distended, and the same fluid was made to pass several times through the organ in the above manner. The total time occupied averaged twenty-five minutes.

B.—Determination of the Lethal Dose of the Circulated Toxin.

A series of guinea-pigs were inoculated with the toxin after its circulation through the isolated liver as above described, this toxin having been first freed from solid particles by centrifugalisation.

(*a*) 0·1 c.c.—Result nil.
(*b*) 0·2 c.c.—Nil.
(*c*) 0·3 c.c.—Nil.
(*d*) 0·5 c.c.—A slight swelling produced, disappearing after twenty-four hours.
(*e*) 0·7 c.c.—A swelling; well next day.
(*f*) 0·9 c.c.—A swelling; well next day.
(*g*) 1 c.c.—Fairly marked swelling; lost in two days.
(*h*) 1·3 c.c.—A marked swelling followed by an abscess, but final complete recovery.
(*i*) 1·5 c.c.—A large swelling, followed by necrosis and death on the sixth day.
(*j*) 2 c.c.—A large swelling; death on the second day.

This series of experiments shows a great increase of the dose of toxin required to produce lethal result after circulation through the liver as compared with that of the original toxin. This increase is far greater than would correspond to any possible dilution which might have taken place on account of the conditions of the experiment.

C.—Method of Preparation of the Nucleo-proteid Solution from Livers through which Diphtheria Toxin had been circulated.

Diphtheria toxin standardised as described in the note on p. 50 was caused to circulate through an isolated liver for thirty minutes. The liver was well kneaded and then rapidly disintegrated by being ground up with sterilised sand and kieselguhr.[1] The resulting mass was subjected to strong pressure in a filter press and the expressed juice collected. To this was then added a dilute solution of acetic acid in the proportion of 0·5 c.c. of 33 per cent. acetic acid to each 100 c.c. of the liver juice. The resulting precipitate was collected and washed with sodium chloride solution of moderate strength, and lastly with slightly acidulated distilled water. The precipitate was then dissolved in a 0·5 per cent. solution of sodium carbonate, forming a rather viscid liquid, rich in nucleo-proteid.

[1] The temperature being meanwhile kept low by surrounding the containing vessel with a freezing mixture.

D.—Experiments to Determine the Action of this Nucleo-proteid Solution upon Guinea-pigs.

(*a*) 0·1 c.c. injected under skin.—No marked effect.
(*b*) 0·2 c.c. injected under skin.—No marked effect.
(*c*) 0·3 c.c. injected under skin.—Slight local swelling; otherwise nil.
(*d*) 0·4 c.c. injected under skin.—No marked effect.
(*e*) 0·5 c.c. injected under skin.—Swelling; well in thirty-six hours.
(*f*) 0·5 c.c. injected under skin.—Swelling; some necrosis on fourth day; recovery.
(*g*) 1 c.c. injected under skin.—Marked swelling; abscess; animal recovered.
(*h*) 1 c.c. injected into vein.—Rapid death; large vessels full of clot.

The surviving animals were then kept for three days without further treatment, the nucleo-proteid solution being kept in a refrigerating chamber. The first four guinea-pigs then received further injections as follows:—

(*a*) 0·2 c.c.—Result nil.
(*b*) 0·3 c.c.—Nil. (*c*) 0·4 c.c.—Nil. (*d*) 0·45 c.c.—Nil.

After another three days' interval, the same four guinea-pigs were again injected with a freshly prepared nucleo-proteid solution.

(*a*) 0·3 c.c.—Result nil.
(*b*) 0·5 c.c.—Nil.
(*c*) 0·8 c.c. in two portions.—Result nil.
(*d*) 1 c.c. in two portions.—Slight swelling, otherwise well.

The same animals were then kept under observation for seven days, after which all four and a control guinea-pig were injected with 0·1 c.c. of standardised diphtheria toxin.

Result.

Control died on second day.
(*a*) Nil.
(*b*) Slight local swelling; well on second day.
(*c*) Nil. (*d*) Nil.

E.—The next series of experiments was then undertaken to ascertain what amount of toxicity was possessed by the expressed juice from livers through which the diphtheria toxin had been caused to circulate. For this purpose guinea-pigs weighing as nearly as possible 500 grms. were used.

The results obtained were as follow:—
(*a*) 0·1 c.c. under skin.—Result nil.
(*b*) 0·2 c.c. under skin.—Result nil.
(*c*) 0·3 c.c. under skin.—Local swelling; gone on third day.
(*d*) 0·4 c.c. under skin.—Local swelling; gone on fourth day.
(*e*) 0·5 c.c. under skin.—Swelling marked; some local necrosis.
(*f*) 0·75 c.c. under skin.—Swelling, abscess; recovery.
(*g*) 0·9 c.c. under skin.—Swelling, abscess; death on sixth day.
(*h*) 1 c.c. under skin.—Swelling; death on second day.
(*i*) 1 c.c. in peritoneum.—Peritonitis, effusion; death.
(*j*) 0·5 c.c. in vein.—Death; intravascular clotting.

These results appear to show that injections of the centrifugalised liver juice have an action similar to injections of the nucleo-proteid solution, but that they are less readily tolerated.

The animals were then allowed to rest for a week, when the first four guinea-pigs were again inoculated with juice from another prepared liver, with the following results:—

(*a*) 0·3 c.c.—Result practically nil.
(*b*) 0·5 c.c.—Slight swelling; well three days later.
(*c*) 0·6 c.c.—Slight swelling with induration; recovery.
(*d*) 0·75 c.c.—Swelling; recovery by fourth day.

After another week's rest the same four guinea-pigs, together with a control, were injected under the skin with 0·1 c.c. of standard diphtheria toxin, with the following results:—

(*a*) Much swelling; recovery.
(*b*) Much swelling; recovery.
(*c*) Much swelling; recovery.
(*d*) Slight swelling during first day; recovery.
Control.—Dead in fifty hours.

After a further interval of one week the surviving guinea-pigs were injected with 0·3 c.c. of toxin, with a uniformly fatal result.

F.—Experiments to ascertain the effect upon standardised diphtheria toxin of its circulation during thirty minutes through an isolated liver.

In this series the guinea-pigs inoculated all weighed as nearly as possible 500 grms., and they had all been recently fed.

(*a*) Control, received 0·1 c.c.—Normal toxin; result dead in two days.
(*b*) 0·1 e.c. circulated toxin.—Result nil.
(*c*) 0·2 c.c. circulated toxin.—Result nil.
(*d*) 0·4 c.c. circulated toxin.—Result nil, save passing swelling.
(*e*) 0·6 c.c. circulated toxin.—Result swelling; recovery.
(*f*) 0·8 c.c. circulated toxin.—Result swelling; recovery.
(*g*) 1·0 c.c. circulated toxin.—Result swelling; recovery.
(*h*) 1·5 c.c. circulated toxin.—Result swelling; died on fourth day.
(*i*) 1·5 c.c. circulated toxin.—Result swelling; dead by sixth morning.

G.—Experiments upon Cats.

In this series of experiments cats received intravenous injections of peptone solution, the blood being thereby rendered incoagulable. Their livers were then isolated from the general circulation in the manner already described, cannulæ being inserted in the portal and suprahepatic veins, and also into the gall bladder. The common bile duct was then ligatured, and the animal rapidly bled to death. The blood was collected and mixed with four times its volume of diphtheria toxin. The portal circulation was then washed out with Ringer's fluid, after which the mixture of blood and toxin was caused to circulate slowly through the portal vessels during a period of twenty minutes by means of the artificial circulation apparatus. The fluid was collected and utilised for the following further experiment with the view of ascertaining its lethal action:—

Guinea-pig, weight 300 grms., 0·2 c.c. injected under skin.—Result slight swelling.

Guinea-pig, weight 260 grms., 0·25 c.c. injected under skin.—Result slight swelling.

Guinea-pig, weight 295 grms., 0·2 c.c. injected under skin.—Result swelling; gone on third day.

Guinea-pig, weight 300 grms., 0·35 c.c. injected under skin.—Result swelling; well by third evening.

Guinea-pig, weight 305 grms., 0·4 c.c. injected under skin.—Result swelling; dead on seventh day.

Guinea-pig, weight 330 grms., 0·5 c.c. injected under skin.—Result swelling; dead in four days.

The effect of the circulation through the liver as above described will be plainly seen by comparison with the results obtained by the injection of the same mixture of blood plus toxin into two guinea-pigs before circulation.

Guinea-pig, weight 300 grms., 0·2 c.c. injected under skin.—Dead in three days.

Guinea-pig, weight 310 grms., 0·15 c.c. injected under skin.—Local swelling; ultimate recovery.

The bile secreted during the circulation time was collected, and amounted to 5 c.c. Its action was tested as follows:—

Guinea-pig, weight 350 grms., 1 c.c. bile injected under skin.—Result nil, save local swelling.

Guinea-pig, weight 250 grms., injected with 1 c.c. of bile which had been mixed half an hour previously with 1 c.c. standardised diphtheria toxin. Death ensued on the fourth day after injection, with local swelling and necrosis.

The remaining bile was kept in a cold storage-room for three days. It was then injected into another guinea-pig, but was found to have become contaminated with *Bacillus coli*.

To recapitulate the conclusions at which we have arrived—(1) During the circulation of diphtheria toxin through the liver, its lethal action is greatly diminished. This diminution occurs whether the toxin be mixed with an indifferent fluid or with blood. (2) The bile from such a liver has a slight antitoxic action. (3) The juice from such a liver has also a slight antitoxic action. (4) Nucleo-proteids separated from the liver juice have a marked antitoxic action.

These experiments appear to show that not only does the liver possess the power of diminishing the lethal activity of diphtheria toxin, but that it probably forms an antitoxin. This power does not depend upon the blood present in the liver, but on the liver tissue itself. The behaviour of the liver in lessening the toxic power of diphtheria toxin is similar to that which it exerts in ordinary digestion in lessening the toxic action of peptones. We say therefore that our experiments tend to support the view advanced by one of us in his address at Moscow, that "immunity, natural or acquired, is nothing more than an extension to the cells of the tissues generally of a power which is constantly exercised during digestion by those of the intestine and liver."

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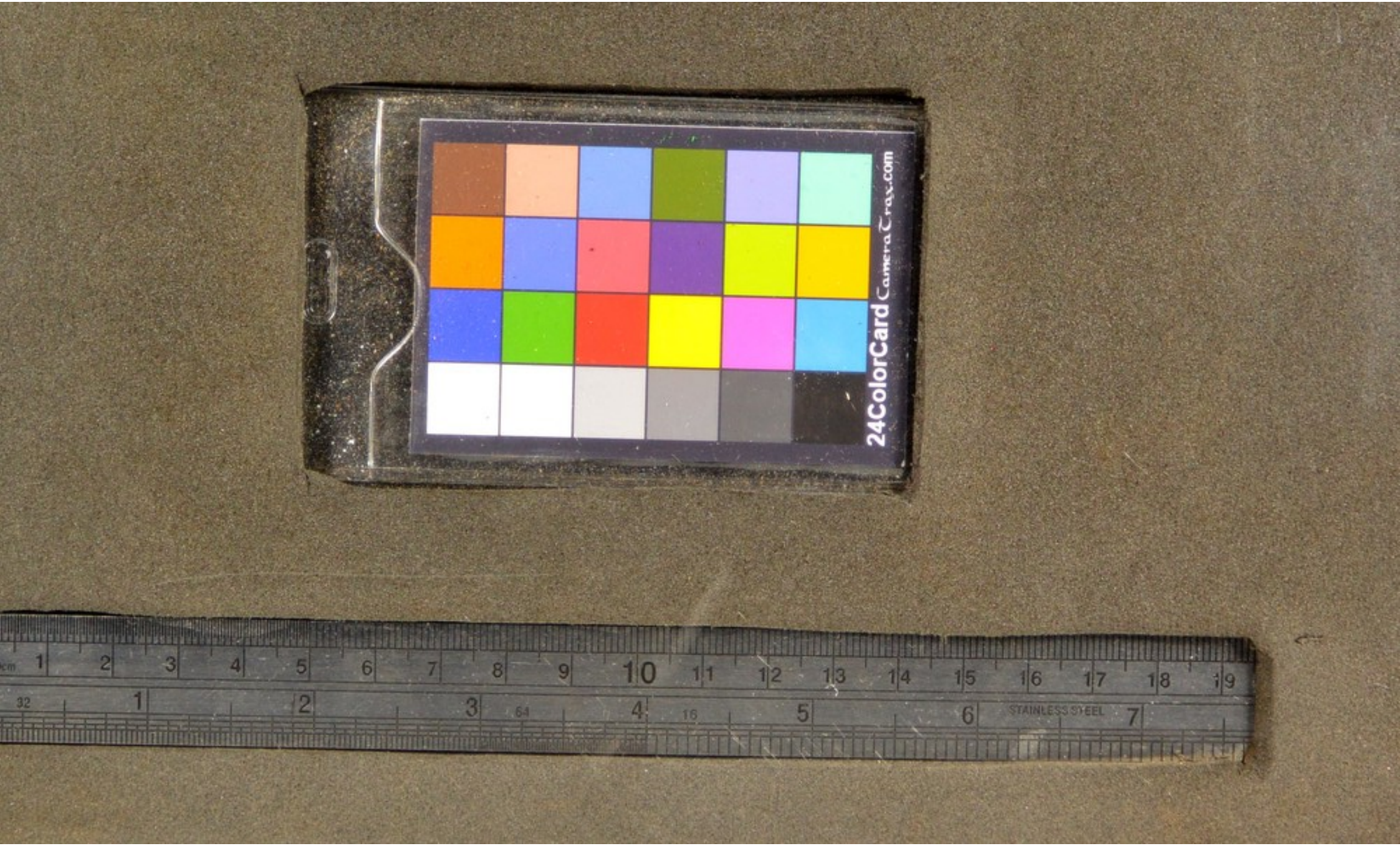
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