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THE VARIABILITY IN RABBITS USED FOR THE ASSAY OF INSULIN.

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THE question whether insulin deteriorates in the tropics, owing to the heat to which it is subjected to when passing through the Red Sea or during the hot weather, was first brought forward by Major E. C. Taylor, I.M.S., in 1923. More recently, in a letter in the *Indian Medical Gazette*, it was suggested that this apparent deterioration may have something to do with the difference in breed of rabbits used in these assays. The more experience one gains in these pharmacological assays, the more one realizes the importance of

the variations in the different individuals under test, or even in excised tissues taken from the same species.

Dale in 1923 first pointed out that the excised uteri of guinea-pigs varied considerably in their reactions to the different test drugs; thus the pregnant uterus is the most sensitive and most unreliable, then the multiparous, and the virgin uterus is the least sensitive and most reliable. The most consistent results are given by the virgin uterus of a guinea-pig weighing about 250 grms., whilst the long thin and fibrous type of virgin uterus responded very feebly to these stimuli.

Recently Acton and Chopra (1924) have shown that the various endocrine glands that stimulate the sympathetic nervous system play a large part in these individual variations. In 1911 and 1912 Acton and Knowles noticed that, during the course of immunising goats against cobra venom, the lighter the colour of the animal the more were they susceptible to cobra and Russell's viper venoms. Most of the white goats succumbed during the attempt at immunisation by gradually increasing doses of venom. The brown goats gave a good yield of serum, whilst the black goats, although they tolerated the venom well, gave a poor yield of antivenene and the blood was often hæmolysed. The best animals for producing these anti-sera are horses of middle age, grey, or chestnut in colour, with good bone. The white Arabs are sensitive to these poisons, so that immunisation has to be carried out more carefully, but the animal eventually yields a good serum.

McLeod and Banting (1924) pointed out that adrenalin produces hyperglycæmia when injected into a rabbit in 1 c.c. doses. Acton and Chopra (1924) showed that an excess of adrenalin tends to inhibit the action of many of these bases.

We were requested from Simla to test the different brands of insulin to see whether any deterioration had taken place in India. The first point that was necessary to study was to see how far these individual variations in the blood sugar by insulin depended on individual variations in these animals, before we could attempt any assay methods. We therefore determined to test (1) the effects of the colour variations of these animals, and (2) the antagonism exerted by adrenalin towards insulin. The results of our experiments on these two factors are as follows:—

The effects of colour variations in the rabbits tested by insulin.—Three types of rabbits were used, the albino Himalayan, the piebald Himalayan, and the grey Belgian hare rabbit. As we were unable to obtain rabbits of the standard weight of two kilograms, we kept our doses of insulin fixed at three units per kilo body weight, and varied the amount according to the weight of the rabbit. The results of these tests are shown in Table I.

TABLE I.

Shows the effects of 3 units of Insulin per kilo body weight.

Type of rabbit.	Weight of rabbit (grammes).	Rate of dosage.	Actual dose injected, units.	Blood sugar before. %	Blood sugar 2 hours after. %	Percentage of reduction.	REMARKS.
White rabbit ..	1,600	3 units per kilo.	4.5	0.100	0.055	45
Do. ..	1,500	"	4.5	0.118	0.06	50
Do. ..	1,800	"	5.5	0.112	0.056	50
Do. ..	1,010	"	3.0	0.110	0.059	47
Do. ..	1,008	"	3.0	0.110	0.06	46
Brown Belgian hare rabbit.	2,000	"	6.0	0.116	0.05	57
Do. ..	2,100	"	6.0	0.112	0.048	57
Do. ..	1,200	"	3.5	0.108	0.049	55
Do. ..	1,900	"	5.5	0.116	0.05	57
Black and white	1,560	"	4.5	0.120	0.048	60
Do. ..	1,500	"	4.5	0.100	0.036	64	Severe convulsions, rabbit recovered after subcutaneous injection of 0.5 c.c. of adrenalin chloride (1 in 1,000) and 2 grms. of glucose.
Do. ..	1,500	"	4.5	0.110	0.041	63	Hurried respiration. Animal in evident discomfort.
Do. ..	1,008	"	3.0	0.120	0.04	67	Hurried respiration. Shivering, no actual convulsion.
Do. ..	1,100	"	3.0	0.100	0.045	60	Severe convulsion, retraction of the head and extension of hind limbs. Subcutaneous injection of glucose given and the life of the animal was saved with difficulty.

In rabbits the percentage reduction in the blood sugar was estimated at the end of two hours after a dose of three units of insulin per kilo body weight. It varied from 45 to 50 per cent. reduction in the white rabbits. In the brown Belgian hare rabbits it varied from 50 to 57 per cent., and in the black and white rabbits two out of the five animals showed very severe symptoms of hypoglycæmia. Therefore colouration and breed of the rabbits had a distinct effect on whether insulin was able to convert the α , β glucose into the γ glucose.

Adrenalin antagonism.—In two rabbits, a black and white and an albino, the blood sugar was tested before an injection of insulin, and again two hours afterwards. In the first rabbit the percentage reduction was 60 per cent. with severe convulsive symptoms, and in the second one the reduction of blood sugar was 40 per cent. Two days later the same two rabbits were again tested, but before the insulin was given, three injections each of 0.05 c.c. of adrenalin hydrochloride were injected subcutaneously every twenty minutes. This was followed by a subcutaneous injection of three units of insulin. The percentage reduction of sugar in these two rabbits was 8 to 10 per cent. respectively. The results of these tests are

given in Table II. In a third rabbit, black and white, not previously tested, the reduction was 18 per cent.

It will be seen therefore that the 0.15 c.c. of adrenalin injected was capable during an hour's time of almost completely preventing the reduction of blood sugar which should have occurred.

Banting showed that a much larger dose, i.e., 1 c.c. of 1-1,000 adrenalin, was capable of producing hyperglycæmia in these rabbits, and when followed by insulin there was very little alteration in the blood sugar. The adrenalin content of the blood varies a lot in the different animals; for example the white rabbits give a much more marked response than the black ones to this base. It has also been shown that during fright the adrenalin content is markedly increased in an experiment done by us. On two occasions when the rabbit was quiet the blood sugar was 0.186 and 0.175. When the same animal had been frightened the blood sugar on two occasions was 0.249 and 0.301 per cent., respectively, showing that the increase in the adrenalin content was capable of producing hyperglycæmia.

The degree of hyperglycæmia produced by adrenalin.—A small dose of adrenalin, 0.15 c.c., was injected into six rabbits of different colours,

TABLE II.
Antagonism between Adrenalin and Insulin. Effect of Insulin alone.

Type of rabbit.	Colour.	Weight.	Dose of Insulin injected.	Blood sugar before. %	Blood sugar 2 hours after. %	Percentage reduction.	REMARKS.
Rabbit No. 1 ..	Black and white.	1,100 grms.	3 units.	0·100	0·045	60	Severe convulsions. retraction of the head and extension of hind limbs. Subcutaneous injection of glucose given and the life of the animal was saved with difficulty.
Rabbit No. 2 ..	White ..	1,008 grms.	3 units.	0·110	0·006	46

Three injections of 0.05 c.c. Adrenalin were given subcutaneously every 20 minutes, followed by subcutaneous injection of 3 units of Insulin.

Rabbit No. 1 ..	Black and white.	1,100 grms.	3 units.	0·120	0·108	10
Rabbit No. 2 ..	White ..	1,008 grms.	3 units.	0·112	0·104	8
Rabbit No. 3 ..	Black and white.	1,500 grms.	4·5 units.	0·129	0·106	18

and the blood sugar tested before and after the adrenalin. The results are shown in Table III.

TABLE III.

Type of rabbit.	Weight in grammes.	Dose of adrenalin injected.	Blood sugar before. %	Blood sugar one hour after. %	Percentage rise of blood sugar.
Albino Himlayan.	1,800	0·15 c.c.	0·120	0·2	66
Do. ..	1,560	„	0·120	0·2	66
Belgian hare ..	1,290	„	0·106	0·17	60
Do. ..	1,960	„	0·100	0·158	58
Piebald. Black and white	1,460	„	0·112	0·170	51
Do. ..	1,020	„	0·129	0·2	55

Here we see that in the albino rabbits the percentage rise of blood sugar was 66 per cent., whilst in the black and white the rise was only 51 and 55 per cent. Therefore the albino rabbits are the most sensitive to small doses of adrenalin, and consequently a greater rise in the blood sugar occurs. The black and white are the least sensitive and the degree of hyperglycæmia is consequently less. This finding is the opposite to the effects of insulin in these animals of different colour. We can, therefore, state that in albino rabbits the adrenalin content is higher, and therefore it is antagonistic to insulin, and this explains

the difference in susceptibility of the different coloured rabbits under test.

Conclusions.

- (1) In the different rabbits tested by insulin there was a variation in the reduction of the amount of blood sugar after the same dose.
- (2) This variability is closely correlated to the colour of the animal tested; the albinos are the least affected and the black and white have convulsive symptoms due to the hypoglycæmia.
- (3) Small doses of adrenalin inhibit the action of insulin by causing hyperglycæmia.
- (4) The adrenalin content can be markedly increased by fright and exercise.
- (5) The colour variations are closely associated with the adrenalin content in the blood of these animals.

SOME OBSERVATIONS ON THE TOXICITY OF EMETINE.

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In a previous paper⁽¹⁾ we discussed in detail the pharmacological action and therapeutic properties of emetine, and pointed out that it was a potent drug and that its dosage and the length of