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Studies of the influence of various dietary conditions on physiological resistance. I. The influence of different proportions of protein in the food on resistance to the toxicity of ricin and on recuperation from hemorrhage.¹

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It was planned to compare, in these experiments, the behavior of two sets of dogs under variously induced pathological conditions. The experiments were conducted on two pairs of dogs, each pair being kept under identical conditions, so far as they could be controlled, except with respect to the food. One animal of each pair was given *liberal* amounts of protein in the daily diet, the other received *barely sufficient* protein to provide for the necessary nitrogenous metabolism, the remaining ingredients of the food for each animal being uniformly equal to the daily amounts ordinarily given per kilo, in this laboratory, to perfectly healthy dogs. No attempt was made to maintain equal caloric values in the diets. In each experiment the animal was fed on a diet of hashed lean meat, cracker meal and lard, the meat being gradually increased or diminished to a high or low plane, according to the plan in each case and before the particular pathological condition was induced.

It was a part of the plan of the work to keep the animal in each instance on a high or low plane of protein nutrition for a *considerable period* before the pathological phase was brought into the experiment. Such a course is not only desirable but essential, for if, as has been claimed, a diet rich in protein exercises a dele-

¹ This study was begun during the summer of 1905, at Dr. Gies' suggestion and has been carried forward from time to time under his direction and with the aid of a grant from the Rockefeller Institute. I am also indebted to Dr. Flexner for important suggestions.

The work has been frequently interrupted by researches in other directions and has been beset by unusual experimental difficulties. Although planned to be the first of a series of investigations, in point of publication it is the second from this laboratory on the general subject stated above. See Dissertation by Welker, Columbia University, 1908. It is Dr. Gies' intention to continue investigation along these lines.

terious influence on the animal organism, that effect is not ordinarily an immediate one, but rather the slow perversion of organic function induced by chronic misuse. Again, if benefit results from a special diet under any condition, time may be required to make that benefit clearly distinguishable. The dogs used in these experiments were under daily observation for periods of at least two months and in several cases for four months before inauguration of the pathological phase.

In testing the physiological resistance of the dogs in these experiments, use was made of two methods: (1) hypodermic injection of the toxin, ricin; and (2) the withdrawal of definite amounts of blood from one of the large arteries. Ricin was selected because it produces some symptoms which are analogous to those of acute infections, namely, fever, cardio-vascular embarrassment and marked prostration. As in the case of infections, ricin also causes noticeable stimulation of metabolism, as is shown by the increased elimination of nitrogen and sulfur after its injection. The disadvantages in its use are that immunity quickly arises and that the material is so very toxic that no latitude is permitted for minor individual peculiarities in animals. By experiment 1 milligram per kilo was found to be the maximum non-lethal dose for the commercial sample used.

EXPERIMENTS WITH RICIN.

Six dogs were subjected to inoculation with ricin. Of these, three were on a high plane of protein nutrition, the daily amount of food containing uniformly in each case 1.4 to 2.0 grams of N per kilo of body weight. After inoculation with ricin (1 mg. per kilo), all of these three specially well-fed animals died. A fourth dog was on a medium plane of protein nutrition, 1.1 gram. of N per kilo, and this dog survived the inoculation. Of the two remaining animals of this group, both on a low plane of protein nutrition, one died and one survived. The dog which survived received the protein equivalent of 0.35 gram of N per kilo of body weight during the experimental period and the one that died, 0.37 gram of N per kilo.

In order to test the effect of exercise, the former of these two dogs, after the completion of the above mentioned experiment, was

allowed to run at large in the laboratory for six months (July 17, 1906, to Jan. 10, 1907), no effort being made to collect excretions for analyses. During this time the protein content of the diet was gradually dropped to an equivalent of 0.27 gram of N per kilo. The animal was painfully thin but appeared to be in excellent health. She was lively during the warm months of summer and autumn, but with the advent of colder weather she became less and less active and lost weight, although the laboratory was adequately heated. It finally became evident that the diet must be changed. The portion of meat in the diet was gradually increased, but a diarrhea supervened, which proved fatal.

HEMORRHAGE EXPERIMENTS.

Although hemorrhage is one of the crudest factors for the determination of physiological resistance, it has, nevertheless, the advantage of being a real test, inasmuch as the production of new blood is an exemplary reparative process. Hemorrhage, unlike the injection of ricin, can also be gauged, to some degree at least, to meet the individual resistance of the animal, as I have found in these experiments. The procedure was uniform throughout this part of the work. At the first hemorrhage from each animal, the endeavor was made to take blood equivalent to 4 per cent. of the body weight. After an interval of four days this procedure was repeated; and again eight days after the initial hemorrhage, making three hemorrhages at successive intervals of four days. All operations were conducted under ether narcosis.

Six dogs were experimented on in this manner, three upon high, three upon low, planes of protein nutrition, and four survived. Of the two which died, one had been on a low plane of protein nutrition (0.4 gram of N per kilo) and one on a high nitrogen plane (1.4 gram per kilo). Control animals on more extreme dietary conditions, withstood the effects of greater hemorrhages. Therefore, it is evident, I think, that these two deaths must be assigned to reasons outside the realm of this research. In the case of the dog on the high plane of protein nutrition, the cause is not far to seek; the dog was a collie, apparently pretty well bred, and it is a matter of common knowledge that these dogs have very low resistance. The dog that died on a low plane of protein nutrition was a fox terrier mongrel.

I. *Outlines of the Experiments.*

Dog No.	N of food per kilo, grams.	Nature of the experiment.				Result.
		Ricin.	Hemorrhage.*			
			Per cent. of body weight.			
		1 mg. per kilo of body weight.	1	2	3	
I	0.35	+	...	...	...	Very sick : Survived.
II	1.10	+	...	...	...	Survived.
III	0.37	+	...	...	...	Died.
IV	1.5	+	...	...	...	Died.
V	3.37 —for 1 mo. 0.51†—for 20 ds.	+	...	...	...	Over fed : Died.
VI	0.40	+	...	...	...	Died.
VII	0.44	...	3.3	2.5	...	Died.
VIII	1.57	...	4.0	4.3	3.1	Survived.
IX	1.4	...	3.8	2.3	0.6	Died.
X	0.35	...	4.2	4.0	4.0	Survived.
XI } Twins.	0.81	...	3.6	3.4	3.7	Survived.
XII }	0.23	...	4.2	3.0	2.9	Survived.

* The numerals above the figures for percentage indicate the number of the hemorrhage in the series applied to the dog in question.

† The food was dropped to this equivalent at once after two days of fasting following pronounced gastro-intestinal disturbances.

II. *General Summary.*

Totals (I — XII)	{	High plane of protein nutrition — 6 dogs	{	Survived, 2
				Died, 4
	{	Low “ “ “ 5 dogs	{	Survived, 2
				Died, 3
Ricin experiments	{	High plane of protein nutrition — 3 dogs	{	Survived, 0
				Died, 3
	{	Low “ “ “ 2 dogs	{	Survived, 1
				Died, 1
Hemorrhage experiments	{	High plane of protein nutrition — 3 dogs	{	Survived, 2
				Died, 1
	{	Low “ “ “ 3 dogs	{	Survived, 2
				Died, 1

In order to reduce individual idiosyncrasy to its lowest terms the last experiment of the series was conducted on two dogs from

the same litter. These animals were half-breed Scotch terriers, about two years old, that had lived together under the same conditions until they were brought to the laboratory. One was gradually accustomed to a diet containing only 0.2 gram of N per kilo, the other to food containing 0.8 gram of N per kilo. As a matter of further interest the opsonic index of these dogs was several times estimated, using *Staphylococcus aureus* that had been rendered pathogenic to dogs. This index was, and remained, the same for both animals. The final hemorrhages were survived by both animals and, so far as one might estimate, about equally well.

The accompanying tables present an outline of the experiments and show details not mentioned above.