THE EREPTIC POWER OF TISSUES AS A MEASURE OF FUNCTIONAL CAPACITY.

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IN a former paper[1] I showed that the peptolysing ferment erepsin, discovered by Cohnheim[2] in the intestinal mucous membrane, is present in all animal tissues. Its function is not known, but since similar relative amounts of it were found to be present in the tissues of various animals, it must be dependent on the chemical nature of these tissues. Presumably it is likewise related to their functional power, and it is the object of this paper to inquire into this relationship. The data adduced seem to indicate that as a rule the changes of ereptic power which a tissue may undergo vary *pari passu* with its functional capacity, and hence afford a convenient measure of this capacity.

The method of experiment has already been described in previous papers[3]. Stated briefly, it consisted in chopping up the tissue under examination as finely as possible, mixing each gram of it with 2 c.c. of glycerin, and estimating the amount of erepsin in the filtered glycerin extract three weeks later. The extract was allowed to act upon 40 times its volume of 2·5 % Witte's peptone at 38° C. in presence of ·1 % Na_2CO_3 and toluol, and the time required by it to hydrolyse 20 % of this peptone determined by a colourimetric method dependent on the biuret test. From the time so determined the ereptic value of the extract was directly deduced, as, within certain limits, the one varies inversely as the other.

THE CHANGES IN THE EREPTIC POWER OF TISSUES DURING GROWTH.

The ereptic power of tissues was found to vary considerably during the growth of an animal, especially during intra-uterine development and

[1] Vernon. This *Journal*, XXXII. p. 33. 1904.
[2] Cohnheim. *Zeit. f. physiol. Chem.* XXXIII. p. 451. 1901; XXXV. p. 13. 1902.
[3] Vernon. *Ibid.*, and this *Journal*, XXX. p. 330. 1903.

the first few days of post-natal existence. Data in support of this statement were obtained in the case of three different animals, but they are necessarily somewhat incomplete. The ereptic values obtained with guinea-pigs are given in the accompanying table. It will be seen that

Age of guinea-pig ... Weight ...	fœtal 37 gms.	0 day 57 gms.	8 days 81 gms.	31 days 238 gms.	3 months 385 gms.	adult 575 gms.	adult 765 gms.	adult 960 gms.	Means of values from 8 days onwards
Kidney	1·8	6·8	8·0	8·0	13·3	5·9	8·5	10·0	8·9
Liver	1·6	2·6	3·3	2·0	5·6	1·8	3·4	3·9	3·3
Cardiac muscle	·48	·67	·70	1·4	4·0	1·1	1·5	1·1	1·6
Skeletal muscle	·56	·79	·90	1·1	·54	·35	·48	·53	·65
Brain	·45	·26	·64	·69	·80	·80	·60	·85	·73

they concern only five tissues, viz., the kidney, liver, cardiac muscle skeletal muscle (cut from the thigh muscles), and brain (including the cerebellum). The results are arranged according to the age of the animal, those in the first column being obtained with the combined tissues of three fœtal guinea-pigs. These animals weighed 34, 35, and 42 grams respectively, or about two-thirds as much as a guinea-pig which was killed within an hour of birth, and the data of which are given in the next column. The guinea-pigs killed after 8 and 31 days were from the same litter as the animal killed at birth, and they were the offspring of the guinea-pig weighing 575 grams, the data of which are given in another column of the table. Taking first of all the ereptic value of the kidney extracts, we see that this was smallest in the fœtal animals, it being only about a fourth as great as that in the animal killed at birth. This value, again, is smaller than that of the 8 day guinea-pig, but from this point onwards there was no definite increase of ereptic value with the growth of the animals. Thus the mean of all the values from 8 days onward is 8·9, whilst the mean of the three values obtained with adult guinea-pigs is 8·1. The three month guinea-pig showed an exceptionally high value, not only for its kidney but also for its liver and cardiac muscle, but judging from the results obtained with rabbits this was only a matter of chance, and cannot be taken to indicate that the ereptic power of the tissues reaches a maximum with half-grown animals, and falls away again as they reach maturity. Accordingly, for purposes of comparison, a mean of all the values from 8 days onward has been taken for each tissue, and these means are given in the last column of the table. The mean kidney value is about five times greater than the corresponding fœtal value, but in the case of the other tissues the difference is not so marked. Thus with the liver the mean value is only about twice as great as the fœtal value, and with cardiac muscle about three times greater, but with skeletal muscle there is little or no change accompanying growth. Thus the fœtal value, though slightly less than the mean, is greater than several of the values obtained with adult guinea-pigs. With brain tissue there is a slight increase of ereptic power accompanying growth, the values obtained with fœtal and 0 day guinea-pigs being on an average only half the mean.

The values obtained with rabbits are even more irregular than those obtained with guinea-pigs, but as a whole they point to the same conclusions. The values in the first column were obtained with the

Age of rabbit... Weight...	fœtal 4·0 gms.	4 days 76 gms.	20 days 181 gms.	31 days 250 gms.	43 days 583 gms.	3 months 1920 gms.	5 months 1980 gms.	adult 2300 gms.	adult 2430 gms.	adult 3300 gms.	Means of values from 20 days onwards
Kidney	—	2·4	9·8	14·3	7·5	6·0	3·6	18·6	20·0	7·3	10·9
Liver	·23	1·0	2·4	3·1	2·1	3·4	1·5	1·6	3·5	1·1	2·3
Cardiac muscle	—	1·4	·95	2·5	1·6	2·3	·89	·34	2·3	·33	1·4
Skeletal muscle	—	·57	·73	1·3	·35	·22	·19	·24	·60	·27	·46
Brain	·03	·41	·57	·75	·50	·56	·14	·42	·80	·18	·49

combined tissues of two fœtal rabbits, which together weighed only 8 grams. Hence the only tissues obtainable in sufficient amount for a ferment estimation were the liver and brain. They proved to be extraordinarily poor in ferment, the liver containing a tenth the mean amount, and the brain only a sixteenth. Comparing these results with those obtained with the more fully grown fœtal guinea-pigs, and with the guinea-pig killed at birth, we may justifiably conclude that the ereptic power of an embryo's tissues is at a minimum in the earliest stages of development, and that it increases steadily during intra-uterine growth, and for the first few days of post-natal existence.

As regards the other rabbit values we see that the kidney and liver tissues of the 4 day rabbit contained very much less erepsin than those of the 20 day rabbit, but that from this point onwards there was no definite increase. The kidney values, in fact, were extraordinarily irregular, that of the 5 month rabbit being only a sixth that of one of the adult animals. The liver values were more constant, but those both of cardiac and skeletal muscle showed considerable irregularities. Moreover, these muscle values showed no definite variations at all with growth of the animals, the tissues of the 4 day rabbit containing the average amounts of erepsin. The brain value of the 4 day rabbit was slightly smaller than the average, but practically speaking one may say that only the kidney and liver showed a distinct increase of ereptic power during post-natal growth.

The data obtained with cats and their offspring, though fewest in number, afford the best proof of all as to the increase of ereptic power with growth. The figures given in the last column of the table are the means of 8 to 10 values obtained with adult cats (for the individual

Age of cat ... Weight ...	fœtal 31 gms.	0 day 109 gms.	6 days 124 gms.	adult 2720 gms.
Kidney	3·1	2·8	4·4	11·6
Liver	·64	·73	2·0	3·6
Cardiac muscle	·73	·37	·68	·95
Skeletal muscle	·36	·31	·29	·56
Brain	·52	·26	·31	1·03
Intestinal mucous membrane	—	3·4	11·8	15·6

values see the next section), and compared with these we see that the values for the 0 and 6 day kittens, as well as those for the fœtal animals, are in all cases much smaller. The fœtal values, obtained with the combined tissues of two animals, are in all but one instance greater than the corresponding values of the kitten killed at birth. However, the renal and hepatic tissues of the 6 day kitten—which was of the same litter as that killed at birth—show a marked increase of ereptic power. The skeletal muscle and brain show no change, but the values are distinctly smaller than the average adult values. The more gradual advance of ereptic power with growth is probably connected with the fact that the kitten is much slower in attaining maturity than the young guinea-pig or rabbit. Thus the guinea-pig attains sexual maturity after 2 months, the rabbit after 6 months, but the cat not for 12 months.

At the bottom of the table are given the ereptic values of the mucous membrane scraped from the whole length of small intestine. It will be seen that these values show a three or fourfold increase during the first six days of post-natal existence. In fact the data suggest that the ereptic power of the tissues is intimately bound up with their functional capacity. During intra-uterine existence the intestinal mucous membrane would scarcely be functioning at all, as the nutriment would be entirely supplied by the maternal blood. Immediately after birth, however, the intestine would have to digest and absorb the maternal milk, or would be in full functional activity. There would accordingly be a greater increase of function in the intestinal mucous membrane than in the liver and kidneys, as these organs would have been carrying on their activities to a limited extent before birth of the fœtus. Still, the work that they were doing would be relatively much smaller than that of the heart, which would be functioning almost as actively in the embryo as in the new-born animal. We find, accordingly, that the ereptic value of the cardiac muscle undergoes comparatively little increase after birth. At least this is the case in the cat and rabbit, though apparently not so in the guinea-pig. In view of the great irregularities to which the individual values are liable, however, this apparent contradiction may not be a real one. The practical constancy in the ereptic value of skeletal muscle during extra-uterine growth is somewhat unexpected, but it should be noted that this constancy applies only to hutch animals, which use their muscles comparatively little at any time. In the more active cat we find that the muscles of the adult contain nearly twice as much ferment as those of the young kitten.

The above results seem at first sight to contradict the conclusions of Schlesinger[1], who found that in new-born rabbits the autolysis of ground-up liver tissue was maximal, whilst in 8 day rabbits it was considerably greater than in older animals. The probable explanation lies in the fact that the tissues of young animals must be much more fragile and easily digestible than those of older ones, and so would more readily undergo autolysis in spite of a deficiency of ferment.

In remarkable agreement with these conclusions concerning erepsin are the results of Batelli and Stern[2] upon the hydrogen peroxide decomposing ferment, catalase. Working with the guinea-pig, these authors found that certain tissues as the liver and kidney showed a rapid increase of ferment during embryonic development and the first few days after birth, but that subsequent to the 7th day there was little or no further increase. At this period the liver contained five times more ferment than that of the new-born guinea-pig, whilst the kidney contained twice as much. The other tissues examined, viz. the spleen, lung, muscle, and brain, showed only a slight increase of ferment with growth.

THE EFFECT OF DIET ON EREPTIC POWER.

To investigate the effect of diet upon ereptic power three cats were kept on a purely meat and fat diet for periods of 11 to 29 days, whilst three others were kept on a bread and milk diet for periods of 14 to 46 days. Extracts were then made, not only of the five tissues mentioned

[1] Schlesinger. *Hofmeister's Beitr.* IV. p. 87. 1904.

[2] Batelli and Stern. *Archivio di Fisiologia,* II. p. 471. 1905.

previously, but of the pancreas and spleen, and of the mucous membrane of the alimentary canal. The stomach and intestine were washed out with a stream of water, and were hung up to drain for half-an-hour before the mucous membrane (and much of the submucosa) was scraped off. The first six inches of the small intestine were regarded as duodenum, whilst the remainder was divided into two equal parts, and regarded as jejunum and ileum. Extracts of the mucous membrane were found to gain their maximum activity earlier than extracts of other tissues, so they were always tested after being kept 12 days instead of 21 days.

The results obtained with these dieted cats are given in the accompanying table. As the individual values are somewhat variable, it is simplest to compare the mean results, and the ratios between these means are given in the last column of the table.

	Meat diet for			Mean meat value	Bread and milk diet for			Mean bread value	Ratio of meat to bread value
Tissue	11 days	21 days	29 days		14 days	20 days	46 days		
Kidney	10·5	8·0	10·1	9·5	16·0	6·9	8·1	10·3	·9
Pancreas	8·7	5·7	4·0	6·1	4·0	7·3	·7	4·0	1·5
Spleen	7·0	7·0	3·6	6·2	6·5	1·3	2·7	3·5	1·8
Liver	3·5	2·6	4·1	3·4	1·5	2·6	1·7	1·9	1·8
Cardiac muscle	·76	·51	·98	·75	·82	·85	·46	·71	1·1
Skeletal muscle	·85	·40	·40	·55	·39	·59	·33	·44	1·2
Brain	1·14	·78	·89	·94	·62	1·09	1·25	·99	·9
Gastric m. m.	7·4	4·3	2·7	4·8	2·4	4·8	5·6	4·3	1·1
Duodenal m. m.	26·5	17·0	23·9	22·5	14·9	14·7	11·8	13·8	1·6
Jejunal m. m.	15·4	12·5	11·8	13·2	14·3	15·4	14·5	14·7	·9
Ileal m. m.	10·4	12·1	6·8	9·8	12·9	8·9	9·5	10·4	·9
Large intest. m. m.	2·9	1·7	3·6	2·7	6·1	7·7	4·0	5·9	·5

From these ratios it will be seen that as a rule the tissues of the meat-fed cats were somewhat richer in erepsin than those of the bread-and-milk-fed. Still the difference is not at all striking except in the case of the spleen and liver. As regards the alimentary canal, we see that the duodenal mucous membrane of the meat-fed cats was in all cases distinctly richer in erepsin than that of the bread-and-milk-fed, whilst the mucous membrane of the large intestine showed in every case an inverse relationship. Presumably the more quickly digested meat provokes a greater activity in the upper part of the gut than the slowly digested bread, which causes a more evenly distributed activity of the whole intestine, including the large intestine.

In addition to these observations upon specially dieted cats, others were made upon four cats fed upon a mixed diet. From the data given in the table it can be seen that the tissues of these mixed diet cats were as a rule considerably richer in erepsin than those of the specially dieted

Tissue	Mixed diet	Mixed diet	Mixed diet	Mixed diet 12 days	Mean
Kidney ...	13·3	—	9·7	20·0	14·3
Pancreas ...	7·3	—	—	5·6	6·4
Spleen	6·7	—	—	8·5	7·6
Liver ...	4·4	5·1	6·2	4·4	5·0
Cardiac muscle ...	2·3	—	—	·89	1·59
Skeletal muscle ...	—	1·33	—	·21	·77
Brain ...	—	1·54	—	·95	1·24
Gastric m. m. ...	—	—	5·0	2·8	3·9
Duodenal m. m. ...	—	—	33·3	22·2	27·7
Jejunal m. m. ...	—	—	12·9	23·5	18·2
Ileal m. m. ...	—	—	9·9	19·0	14·4
Large intest. m. m.	—	—	2·5	9·2	5·8

cats, the liver and cardiac muscle containing about twice as much ferment, and most of the other tissues about half as much again. This somewhat striking result is not altogether a genuine one, however. Thus three out of the four cats were killed within a few days of being kept in captivity, and only one of them was kept for as long as 12 days upon some of the meat and bread and milk given to the dieted cats. It alone, therefore, is comparable with these cats, for it was found that continued captivity had a distinctly depressant effect upon the ereptic activity of most of the tissues. In the next table are given means of the individual values obtained with the cats which were kept in captivity, one on meat and the other on bread and milk, for 11 to 14 days, 20 to 21 days, and 29 to 46 days respectively. These data show that the ereptic value of the spleen is markedly diminished by confinement, whilst that of the kidney, pancreas, and skeletal muscle is moderately so.

	Means of values for cats fed on			
Tissue	Meat 11 days Bread 14 days	Meat 21 days Bread 20 days	Meat 29 days Bread 46 days	Effect produced by confinement
Kidney	13·2	7·4	9·1	moderate
Pancreas ...	6·3	6·5	2·3	,,
Spleen	6·7	4·1	3·1	marked
Liver	2·5	2·6	2·9	*nil*
Cardiac muscle ...	·79	·68	·72	,,
Skeletal muscle ...	·62	·49	·36	moderate
Brain	·88	·93	1·07	*nil*
Gastric m. m. ...	4·9	4·5	4·1	slight
Duodenal m. m. ...	20·7	15·8	17·8	,,
Jejunal m. m. ...	14·8	13·9	13·1	,,
Ileal m. m. ...	11·6	10·5	8·1	,,
Large intest. m. m.	4·5	4·7	3·8	,,

The values obtained with the cat kept on a mixed diet for 12 days are in most cases distinctly larger than those of the cats kept on either meat or bread and milk for a similar period, and so there is little doubt that a mixed diet is more conducive to ereptic activity than an unmixed one. In any case the effect of diet is not a great one. The ereptic power of the tissues seems to be a fairly constant property which is only to a slight extent affected by considerable changes in the diet of the animal. This conclusion seems to be in contradiction to the results obtained by Lane-Claypon and Schryver[1] concerning the effects of previous feeding and fasting on autolysis. These observers determined the rate of autolysis of minced liver and kidney tissue during the first 24 hours after death, and they found that the tissues of fasting cats underwent a considerably more rapid disintegration than those of well fed cats. This seemed to imply that the former contained a larger amount of proteolytic enzyme, but as Schryver himself suggests[2] it is possible that in the well-nourished animal the action of the autolytic enzyme is to some extent inhibited by the presence of metabolites. Certainly my own observations, not only those described above but others to be referred to in subsequent sections, seem to indicate that whatever may hold for other proteolytic enzymes the erepsin at least is but very gradually influenced by nutritive and other changes. This conclusion is in harmony with that of Batelli and Stern[3] concerning catalase. Thus the tissues of the rat showed no sensible diminution in this ferment as the result of 4 to 8 days' starvation.

THE DISTRIBUTION OF EREPSIN IN THE ALIMENTARY CANAL.

The distribution of erepsin in the alimentary canal affords a striking proof of the connection between ereptic power and functional activity. In addition to the observations upon cats, others were made upon the rabbit, hedgehog and dog, and these are collected in the accompanying table. As regards the small intestine of the rabbit, the first seven inches were taken as duodenum, whilst the remainder, divided into two equal portions, represented the jejunum and ileum. The three feet immediately above the anus were taken as a sample of the large intestine. In the case of the hedgehog the five inches immediately above the anus were taken to represent large intestine.

[1] Lane-Claypon and Schryver. This *Journal*, XXXI. p. 169. 1904.
[2] Schryver. This *Journal*, XXXII. p. 159. 1905.
[3] Batelli and Stern. *Ibid.*

The values given in the table for cat's tissues are means of the eight series recorded in the previous tables. From them it appears that the

Mucous membrane of	Mean cat value	Adult dog	Hedge-hog	Hedge-hog	Hedgehog (hiber-nating)	Mean hedgehog value	43 day rabbit	3 month rabbit	Adult rabbit	Mean rabbit value
Stomach	4·4	—	·8	·8	·5	·7	·8	·2	·05	·4
Duodenum	20·5	14·8	10·6	9·1	12·7	10·8	1·4	5·5	4·5	3·8
Jejunum	15·0	14·3	6·8	8·5	9·3	8·2	4·7	9·3	6·5	6·8
Ileum	11·2	13·3	6·6	8·2	10·5	8·4	9·2	7·0	7·1	7·8
Large intest.	4·7	3·7	2·8	4·4	1·3	2·8	·7	·3	·4	·5

mucous membrane of the duodenum contained about five times as much erepsin as that of the stomach or the large intestine, so we see that the greatest ereptic power was concentrated in the small intestine, or where it would be of greatest service. Moreover it is present in largest quantity in the upper part of the small gut. Still the lower part of the gut contains about half as much as the upper, so the difference in distribution of the ferment is not nearly so marked as that found by Chepowalnikow[1] for enterokinase, and that by Bayliss and Starling[2] for secretin. In the dog, judging from the single series of observations made, the erepsin is almost evenly distributed through the small intestine, but in the hedgehog the duodenal mucous membrane shows a slight excess. The gastric mucous membrane of the hedgehog is peculiarly poor in erepsin, containing on an average only a fifteenth part of that present in the duodenal mucous membrane.

The three animals so far mentioned are all carnivorous or omnivorous. When we pass to the herbivorous rabbit we find considerable differences. The whole of the mucous membrane is much poorer in erepsin, and in the small intestine we find the distribution of the ferment just the reverse of that previously experienced. The ileum is now richest in erepsin, whilst the duodenum is poorest. The reason of this is unknown, but presumably the proteid constituents of the herbivorous diet are so indigestible that most of them have not arrived at the albumose and peptone stage till they arrive at the lower part of the small gut. In connection with this inverted distribution of ferment it will be remembered that the cats fed on a bread and milk diet showed a nearly even distribution of erepsin in their small intestine, whilst those on a meat diet showed an especial richness of ferment in the duodenum.

[1] Chepowalnikow. Abstract in *Maly's Jahresbericht*, p. 378. 1899.

[2] Bayliss and Starling. This *Journal*, XXVIII. p. 325. 1902.

THE EFFECT OF HIBERNATION ON EREPTIC POWER.

In that the tissues of a hibernating animal during its winter sleep are in a condition of greatly lowered activity, they presumably possess diminished functional capacity. One would accordingly expect the ereptic power to be weakened, and such was found to be the case in the tissues of the hedgehog. In the accompanying table are given the values obtained, two series with non-hibernating animals, and two with hibernating. The first hedgehog of which the results are recorded was killed on Nov. 19th, or just before entering into its winter sleep. Its body temperature was normal, but the animal seemed torpid and refused to eat food. Its alimentary canal was nearly empty, and its tissues were loaded with fat. The second hedgehog was killed on July 19th, the day after it was caught. Of the hibernating animals, the first was killed on

	Non-hibernating hedgehogs			Hibernating hedgehogs			
When killed ...	Nov. 19	July 19	Mean	Dec. 22	Feb. 15	Mean	
Weight ...	835 gms.	747 gms.	value	490 gms.	548 gms.	value	Ratio
Kidney ...	9·3	6·5	7·9	3·6	4·0	3·8	2·1
Pancreas ...	3·4	2·5	3·0	2·4	3·6	3·0	1·0
Spleen ...	5·8	4·6	5·2	·82	·59	·70	7·4
Liver ...	2·1	1·7	1·9	·77	·76	·76	2·5
Cardiac muscle	·71	·25	·48	·35	·25	·30	1·6
Skeletal muscle	·29	·20	·24	·16	·33	·24	1·0
Brain ...	·21	·24	·22	·15	·31	·23	1·0

Dec. 22nd, when it had a temperature of 7·5°. All the hedgehogs were killed by allowing them to inhale coal-gas, and so depressed were the vital processes of this animal that it continued to show movements for an hour after exposure to the gas. The summer hedgehog, on the other hand, showed no movement after 20 seconds. The second hibernating hedgehog was examined on Feb. 14th, and was then found to have a temperature of 9·2°. Next day, when it was killed, its temperature had risen to 35°, or it had become warm-blooded in consequence of the previous disturbance. In spite of this, the ereptic power of its tissues had not obviously increased, as far as one can judge by comparing the values with those of the other hibernating animal.

On comparing the mean values obtained with the non-hibernating animals against those obtained with the hibernating, we observe very striking differences. From the ratios given in the last column of the table we see that the kidney and liver of the non-hibernating hedgehogs were more than twice as rich in erepsin, whilst the spleen contained no less than seven times more ferment than was found in the hibernating animals. This remarkable difference of ereptic power seems to indicate that this organ possesses important functions in the active warm-blooded animal.

The remaining tissues investigated, with the doubtful exception of cardiac muscle, show no appreciable response to the condition of hibernation. The pancreas, skeletal muscle, and brain gave the same mean values, whilst the mucous membrane of the alimentary canal was somewhat richer in ferment in the hibernating than in the non-hibernating animal. Thus the last of the three series of hedgehog values given in the table in the previous section was obtained with a hibernating animal, and it will be seen that these data, as compared with those for normal animals, indicate a slight excess of ferment in the small intestine, and a deficiency in the stomach and large intestine. However, the results are not quite comparable, as the alimentary canal of the hibernating animal, being quite free of food, was not washed out with a stream of water before being scraped. That of the non-hibernating animals had to be treated in this way, and it may have lost a certain amount of ferment in the process.

Judging from the whole of the results described in this paper it is probable that the diminution of ereptic power found in some of the tissues of hibernating animals is only gradually acquired after the onset of the winter sleep, whilst the increase which must occur when the animal becomes active again in the spring is likewise a gradual one. Accordingly a sudden change from the cold to the warm blooded condition, such as was experienced in one of the above-mentioned animals, would not affect the ereptic power to any great extent. As regards the apparent absence of response to hibernation in several of the tissues, it should be remembered that both the pancreas and intestinal mucous membrane actively secrete erepsin during the normal digestion of food, and so when in a resting condition they would doubtless store up a supply of this ferment. The total erepsin present in extracts of these tissues, which represents both this secretory erepsin and the true intracellular erepsin, might therefore be larger than in the actively functioning tissue.

THE EFFECT OF DISEASE ON EREPTIC POWER.

The effect of disease on ereptic power was studied in the guinea-pig and rabbit, and also in man. As a rule the tissues were not cut up and extracted till several hours after death; in the case of human tissues, often not for 24 hours. Hence it is necessary to determine to what extent the ereptic value of a tissue changes after death. The accompanying table shows the results obtained with some sheep's liver which

Liver extracted	Ereptic value of extract after 1 day	2 days	4 or 5 days	11 or 12 days	28 or 30 days
Immediately	3·2	3·0	3·4	3·0	2·3
After 12 hours	—	3·1	3·4	3·2	—
,, 1 day	3·1	3·3	3·4	3·3	2·0
,, 2 days	3·3	3·3	3·8	3·4	—
,, 3 ,,	3·3	3·3	4·0	3·4	1·9
,, 4 ,,	3·9	4·1	3·8	3·4	—

was finely minced and pounded in a mortar, and then kept four days in a moist chamber. It began to smell putrid after two days, and had acquired a greenish hue after four days. Every day a portion of the mass was withdrawn, and mixed with twice its weight of a mixture of 6 parts of glycerin and 4 parts of water. The ereptic power of these glycerin extracts was tested from day to day in the usual manner. In that diluted glycerin was used instead of concentrated, we see that the maximal ereptic power was attained very much quicker, it being reached in 4 or 5 days by most of the extracts. The onset of putrefaction, so far from destroying any of the erepsin, seemed to assist in its liberation. Thus we see that the tissue kept 4 days before extraction attained the greatest ereptic power of all, and, moreover, attained it after 2 days' extraction instead of after 4 or 5 days'. This somewhat unexpected result does not hold for all tissues, however. In another experiment the kidneys of a guinea-pig were minced, and a part of the tissue extracted with concentrated glycerin at once. The rest was kept in a moist chamber, and part of it extracted after 3 days, and the remainder after 6 days. The fresh extract attained an ereptic value of 8·5, the 3 day extract one of 6·4, and the 6 day extract one of 4·0. In spite of this somewhat marked deterioration, it is probable that the deterioration of ereptic value in an intact kidney kept for one or even two days would be slight or non-existent, for the previous mincing of a tissue greatly accelerates autolysis and the onset of putrefaction. We may assume, therefore, that in all probability the data to be discussed have been but little influenced by any post-mortem changes in the tissues.

The observations on guinea-pigs are three in number. The first column of figures in the table reproduces the mean values for the tissues of normal guinea-pigs given in a previous table. The next column but two shows the values obtained with an adult guinea-pig which died from some cause unknown, but which had wasted greatly before death.

It weighed only 320 grams, or less than half as much as the three normal adult guinea-pigs examined, which averaged 767 grams. None

Tissue	Normal guinea-pigs 767 gms.	Died 3 days after staphylococcus 577 gms.	Died 6 weeks after staphylococcus 290 gms.	Wasted guinea-pig 320 gms.	Mean value for wasted guinea-pigs 305 gms.	Ratio of normal to wasted value
Kidney	8·9	7·8	2·8	4·5	3·7	2·4
Liver	3·3	3·6	1·8	·95	1·4	2·4
Cardiac muscle	1·6	2·8	·83	·61	·72	2·2
Skeletal muscle	·65	·72	·63	·56	·59	1·1
Brain	·73	·41	·30	·32	·31	2·4

of its tissues except skeletal muscle contained even half as much erepsin as those of normal animals, and two of them, viz. the liver and cardiac muscle, contained only a third as much. The previous column in the table shows the values obtained with a guinea-pig which died 6 weeks after an injection of *Staphylococcus pyogenes aureus.* It had in consequence wasted away rapidly, and at the time of death weighed less than half its original amount. As in the previous case its tissues—again with the exception of skeletal muscle—contained only half to a third the normal amount of erepsin. The mean values obtained with these two wasted guinea-pigs are given in the last column but one of the table, and in the last column are given the ratios between these and the normal values. Excluding skeletal muscle, we see that on an average the normal tissues contained about 2·3 times more erepsin than the wasted ones.

That this great diminution of ereptic power is dependent on the wasting of the animal is well shown by the data given in the second column of figures in the table. These were obtained with a guinea-pig which died 3 days after an injection of *Staphylococcus pyogenes aureus.* This rapid death precluded wasting, and hence we find that the tissues contained practically their normal amount of erepsin.

Upon the rabbit three observations were made, but the results are not so striking as those on guinea-pigs, in that the degree of wasting at the time of death was not so marked. The first column of figures in

Tissue		31 day rabbits 171 gms.	9 week rabbit 360 gms.	Adult rabbit 1900 gms.	Mean diseased rabbit value	Ratio of normal to diseased value
Kidney	10·9	8·7	9·1	4·0	7·3	1·5
Liver	2·3	1·4	2·7	1·2	1·8	1·3
Cardiac muscle	1·4	1·0	1·5	·40	·97	1·4
Skeletal muscle	·46	1·3	·24	·44	·66	·7
Brain	·49	·62	·57	·32	·50	1·0

the table gives the mean values obtained with normal rabbits. The next, the values obtained with the combined tissues of two rabbits which had died from some unknown cause when 31 days old. The weight of these animals averaged 171 grams, or 32 % less than that of a healthy rabbit, of the same litter, which was killed at the same time. The data obtained with this normal animal are given in a previous table, and in comparison with them we find that the kidney, liver, and cardiac muscle of the diseased rabbits were only about half as rich in erepsin. The next column of figures in the table gives the values obtained with a 9 week rabbit which had apparently died from coccidiosis, and the next, those with an adult rabbit which had died of double empyema. This latter animal had wasted a good deal, and the ereptic value of its tissues was as a rule less than half the normal. Taking averages of the three sets of values obtained with diseased rabbits, we see that, compared with the normal values, the ereptic power of skeletal muscle and brain was practically uninfluenced, whilst that of the other tissues was about two-thirds as great.

These observations on guinea-pigs and rabbits are in good agreement with those of Schlesinger[1] on young children. Schlesinger found that the autolytic power of the minced liver tissue varied with the degree of atrophy at the time of death. In children who weighed only 35 % of their normal amount, the autolysis was only half as great as in those who weighed 80 % of the normal.

The next table gives the results obtained with human tissues. They consist in determinations of the ereptic value of small typical samples of the kidney, liver, and cardiac muscle from 19 consecutive post-mortem room cases. For this material, and for the diseased animals above mentioned, I am indebted to the kindness of Professor J. Ritchie. The cases are arranged according to age, and brief notes, copied from the pathological record book, are given concerning the condition of the organs examined.

As far as one can judge from these incomplete data, the age of a patient at death has a distinct influence upon the ereptic power of the tissues. On dividing up the whole of the cases into two nearly equal groups, and taking means, the following results are obtained:

	Mean ereptic value of			
Age	Kidney	Liver	Cardiac muscle	Mean ratio of K. to L.
2½—44	4·2	2·5	·62	1·73
50—76	3·6	4·1	·48	1·07

[1] Schlesinger. *op. cit. supra.*

Age	Sex	Cause of death	Condition of Kidney	Condition of Liver	Condition of Heart	Ereptic value of Kidney	Ereptic value of Liver	Ereptic value of Cardiac muscle	Ratio of kidney to liver value
2½	♂	Septicæmia	minute abscesses	cloudy and fatty	normal	6·2	3·8	1·00	1·6
5	♂	General peritonitis	normal	normal	—	3·5	1·7	—	2·1
17	♂	Pernicious anæmia	fatty	—	aortic incompetence	1·4	·7	·24	2·0
21	♂	Miliary tubercle	normal	cloudy and fatty	normal	6·9	5·2	1·60	1·3
21	♂	Railway accident	normal	normal	normal	6·1	2·1	·82	2·9
31	♀	Pulmonary apoplexy, after ovariotomy	catarrhal	cloudy	—	2·8	1·4	·21	2·0
35	♂	Railway accident	normal	normal	hypertrophied	4·3	3·3	·26	1·3
44	♂	Nephritis	cortex and medulla much diminished	fatty: slightly cirrhotic	fatty infiltration	·86	1·5	·29	·6
44	♀	General peritonitis	diminished cortex: some interstitial	fibro-fatty: nutmeg	hypertrophied	5·4	3·0	·57	1·8
50	♀	Morphia poisoning: previous scirrhus	catarrhal	secondary cancer	slightly fatty	1·7	4·2	·27	·4
54	♂	Pneumonia	cloudy	cloudy	interstitial	4·3	6·8	·25	·6
54	♂	Cerebral hæmorrhage	slightly diminished cortex	slightly fatty	hypertrophied	1·8	1·7	·54	1·1
58	♂	Growth in bladder	fatty	fatty	hypertrophied	8·5	4·8	1·20	1·8
59	♂	Pneumonia	early interstitial	cloudy and fatty	some interstitial	2·6	2·5	·53	1·0
60	♂	Cancer of stomach	normal	cloudy and fatty	normal	6·9	10·5	1·10	·7
60	♂	Septic pneumonia	cloudy	fatty and cirrhotic	slightly fibrosed	1·5	·7	·23	2·1
68	♂	Aortic incompetence	some interstitial	slightly cirrhotic	fatty	1·5	1·3	·27	1·1
74	♀	Cancer of rectum	—	—	—	2·1	1·8	·23	1·2
76	♂	Stricture: cystitis	normal	fatty and nutmeg	normal	4·7	6·5	·19	·7

These data show that as compared with individuals of 44 and under, those of 50 and upwards apparently have somewhat less erepsin in the kidney and cardiac muscle, but distinctly more in the liver. This relative increase in the ereptic power of the liver must, I think, be regarded as genuine. Thus in the last column of the big table are given the ratios between the kidney and liver values in each case, and we see that whilst of the nine younger individuals all but one (and that a case of nephritis) had a larger kidney value than liver value, and six of them a considerably larger value, of the ten older individuals only two had a considerably larger kidney value. The average ratio of kidney value to liver value works out at 1·73 for younger individuals, and 1·07 for older. It is possible, therefore, that the diminished functional power of the kidneys, which is so frequent an accompaniment of old age, causes an increased amount of work to be thrown on the liver, and thereby raises the ereptic power of this tissue.

On classifying the various cases according to the condition of the individual organs, the effect of disease upon ereptic power is strikingly brought out. Taking first of all the kidney, the cases have been split up into four groups. From the table we see that in six of the cases the kidney was said to be normal, and these, grouped together, give a mean ereptic power of 5·4 for the tissue. In two cases the kidney showed a catarrhal condition, in two others cloudy swelling, and in two others some fatty degeneration. These have all been grouped together, as showing only a slightly diseased condition, and they give a mean ereptic value of 3·4. Then in four of the cases there was some interstitial nephritis accompanied by diminished cortex, and these had a mean ereptic value of 2·8, or not much more than half that of the normal cases. Finally, the single case of advanced nephritis, in which the medulla was diminished and the cortex much diminished, gave an ereptic value of only ·86, or a sixth the normal. Clearly, therefore, in the kidney the more advanced the disease the less and less becomes the ereptic power of the tissue.

In the liver the relation between ereptic power and disease is not shown so strikingly as in the kidney. In only three of the cases was the liver said to be normal, and these gave a mean ereptic value of 2·4. There were two cases of nutmeg liver, and eight cases in which the liver showed cloudy swelling accompanied by more or less fatty infiltration and degeneration. These ten cases may be classed together as showing a slightly diseased condition, and they give a mean ereptic value of 4·6. This value is considerably greater than that of normal hepatic tissue, but the number of normal cases was obviously too small to give a reliable result. Finally, in three instances the liver was cirrhotic, the two instances of slight cirrhosis giving a mean ereptic value of 1·4, and that of more marked cirrhosis one of ·7.

As regards cardiac tissue, the five cases in which it was said to be normal gave a mean ereptic value of ·94. In five cases the heart was hypertrophied, and the cardiac muscle then had a mean ereptic value of ·56. This depreciation from the value of normal muscle is greater than one would have expected, as presumably the hypertrophied muscle was functioning quite as vigorously as the normal muscle. In six cases the cardiac muscle showed fatty infiltration or degeneration, or interstitial myocarditis, or some fibrosis. In these cases of definite degeneration of the cardiac muscle the mean ereptic value was only ·32, or about a third that of normal cardiac tissue. In all three of the tissues investigated, therefore, we see that a pathological condition is accompanied by a loss of ereptic power, this loss being—as far as the limited number of observations can indicate—more and more marked the more advanced the disease.

General conclusions.

Before formulating any general conclusions concerning the tissue erepsins, it is expedient to compare the mean ereptic values of the various tissues of different species of mammals. This has been done in the accompanying table. The data given for the kidney, liver, and cardiac tissue of man are the means of determinations made upon the

Animal ... ... No. of observations ...	Cat 8 to 10	Hedgehog 2	Man 2	Rabbit 8	Guinea-pig 7
Kidney	11·6	7·9	5·2	10·9	8·8
Liver	3·6	1·9	2·7	2·3	3·4
Cardiac muscle	·95	·48	·54	1·4	1·8
Skeletal muscle	·56	·25	(·18)	·46	·66
Brain	1·0	·23	(·27)	·49	·68

tissues of two healthy individuals killed by accidents. The values for skeletal muscle and brain, which are given in brackets, were obtained with the tissues of the diseased individual, aet. 68, referred to in the previous table, and are almost certainly abnormal. The data for the hedgehog are the means of the values obtained with the two non-hibernating animals.

On comparing these mean values, one is at once struck by their similarity. Though the animals included in the table consist of carnivora, omnivora, and herbivora, yet the extreme values of a tissue such as the liver vary only from 1·9 to 3·6. The values for the other tissues vary somewhat more, but having regard to the comparatively small number of observations made, and the great differences in the conditions of life of the animals examined, one is led to conclude that, given similar conditions, the average ereptic value of a tissue may be roughly constant through a wide range of the mammalian kingdom. This constancy is striking in comparison with the considerable variations shown by the intracellular ferment catalase. Thus Batelli and Stern[1] have found that animals of different species give widely diverging results. For instance, the kidney and the blood of the guinea-pig and rabbit liberate similar amounts of oxygen, but the liver of the guinea-pig was found to liberate 16 times more oxygen than that of the rabbit. Again, in the pigeon the liver liberated 106 times more oxygen than the blood, but in the adder, the blood 3 times more than the liver.

Quantitative comparisons with the tissues of different animals do not seem to have been made for other intracellular ferments, but Hedin and Rowland[2] determined the rate of autolysis of the juice pressed from the kidney, liver, and heart muscle of the calf or ox, and of the dog. They found that for both animals the kidney juice showed the most rapid autolysis, and the heart muscle juice the least (*i.e.* a similar relationship to that found for erepsin), and that the relative rates of autolysis were similar for the corresponding tissues of the two animals, so far as the results were comparable. The juice must therefore have contained similar amounts of the lieno-β-protease ferment[3], *i.e.* of the proteolytic ferment which acts in an acid medium. Probably, therefore, the various intracellular proteolytic ferments, whether those acting in an acid medium or an alkaline, those acting chiefly upon native proteids or their hydrolytic products, form a fairly constant constituent of each individual tissue, for they are bound up with, and form part of the "biogens" of the tissue protoplasm, and are intimately concerned with their various metabolic processes. We accordingly find that tissues such as the liver, spleen, kidney, and pancreas, which are specially concerned in the maintenance of the general nutrition of the body, *i.e.* in the processes of digestion and excretion of the food material, are rich in erepsin and protease, whilst other tissues, such as muscle and brain,

[1] Batelli and Stern. *Op. cit.*

[2] Hedin and Rowland. *Zeit. f. physiol. Chem.* xxxii. p. 531. 1901.

[3] Hedin. This *Journal*, xxx. p. 155. 1904.

which have no special concern in these particular processes, are poor in erepsin and protease.

SUMMARY.

The erepsin in the tissues of the guinea-pig, rabbit, and cat was found to increase considerably during intra-uterine development and for the first few days of post-natal existence, but during subsequent growth it remained constant. The kidney and liver values showed the most change, they increasing in some cases five- or even ten-fold, whilst those of cardiac and skeletal muscle showed the least.

The ereptic power of most of the tissues is not greatly affected by diet. The spleen and liver of cats fed on meat for 11 to 29 days contained nearly twice as much erepsin as the corresponding tissues in cats fed on bread and milk for 14 to 46 days, but the other tissues were but little affected.

The distribution of erepsin in the mucous membrane of the alimentary canal is very unequal. In cats fed on meat or a mixed diet, the duodenum is about five times richer in ferment than the stomach. The jejunum is about two-thirds as rich as the duodenum, and the ileum half as rich. Feeding on a bread-and-milk diet causes a considerable diminution in the duodenal ferment, and an increase in that of the large intestine. In the herbivorous rabbit the mucous membrane of the alimentary canal is much poorer in ferment than in the cat, dog, or hedgehog, and the duodenum, so far from being richest in ferment, contains only about half as much erepsin as the rest of the small intestine.

Hibernation has a considerable influence on ereptic power. The kidney and liver of hibernating hedgehogs contained on an average less than half the erepsin present in the corresponding tissues of non-hibernating animals, whilst the spleen contained only a seventh as much ferment. Skeletal muscle and brain were uninfluenced by hibernation.

Disease, if accompanied by wasting, greatly reduces the ereptic power of the tissues. In two guinea-pigs which were less than half their normal weight at death, the ereptic value of the kidney, liver, heart, and brain was 2·3 times smaller than the normal. Another animal, which died three days after an injection of *Staphylococcus*, showed no loss of ereptic power, as there was no time for wasting.

In man it was found that individuals of 50 and over, as compared with those of 44 and under, showed increased ereptic power in the liver, but a decreased one in the kidney. Taking individual organs, it was found that as a rule the more advanced the disease the less and less became the ereptic power. For instance, the kidney when normal had an average ereptic value of 5·4; when showing cloudy swelling or fatty degeneration, one of 3·4; and when affected with interstitial nephritis, one of 2·4.

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