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Ketosis Following Administration of Adrenal Cortex Extract.

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Following adrenalectomy the ketosis incident to the metabolic disturbances of fasting,[1] pancreatectomy,[2] phloridzin administration,[3] pregnancy[4] and administration of anterior pituitary extracts[1] does not occur. The mechanism by which the removal of the adrenals abolishes ketosis is not clear. If it is dependent upon a factor which operates through the adrenal cortex,[3] and the production of ketosis by certain anterior pituitary extracts is obviously such a stimulus, either the presence of the adrenal cortex hormone in the organism is necessary or this tissue is stimulated to produce the ultimate ketosis factor. It seemed that the effect of adrenal cortex extract on ketosis might throw some light on this point, although not necessarily proving what mechanism is involved. Evans[3] was unable to increase the urinary excretion of sugar and nitrogen in phloridzinized adrenalectomized animals by injection of a cortical adrenal extract. We have had similar results with one commercial extract ("Eschatin," Parke, Davis & Co.) which we used, but our dosage was high and some of the difficulties were definitely due to the phenol content (0.1%) of the preparation. With our own preparation we obtained indefinite results but another commercial product (Adrenal Cortex Extract, Wilson & Co.) which we have found more potent than either of these in maintaining the life of bilaterally adrenalectomized rats without salt therapy has influenced the ketosis of fasting rats quite definitely. The first adrenal cortex extract (Wilson) which we used contained no preservative. Later supplies contained 1:100,000 sodium ethyl mercurithiosalicylate (known under the trade name of "Merthiolate") but this preservative did not appear to have any effect on our results. Typical data are presented here.

The necessary information about each experiment is given in Table I. The rats were removed from the stock colony, where they

1 MacKay, E. M., and Barnes, R. H., *Am. J. Physiol.* In press.

2 Long, C. N. H., and Lukens, F. D. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 743.

3 Evans, G., *Am. J. Physiol.*, 1936, **114**, 297.

4 MacKay, E. M., and Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 682.

TABLE I.

Excretion of Ketone Bodies in mg. Per Rat Per Day.

No.	Initial body wt., gm.	Day of Fasting 1	2	3	4	5
Experiment 1 (Adult female rats 500-600 days old)						
Control Group						
1	241	16.8	17.3	14.9	1.4	2.5
2	227	2.5	8.1	38.6	11.4	2.4
3	216	4.6	15.3	34.9	13.8	4.9
Av.	228	8.0	13.6	29.0	8.9	3.3
Adrenal Extract Group			(4 cc.)	(4 cc.)	(2 cc.)	
4	235	2.8	75.4	154.0	121.0	140.0
5	234	2.2	16.2	136.0	63.0	6.9
6	225	3.1	61.2	150.0	94.6	113.0
Av.	231	2.7	50.9	146.6	92.9	83.3
Experiment 2 (Adult male rats 240-300 days old)						
Control Group						
1	250	1.6	0.7	1.2	0.7	6.6
2	206		1.6	0.2	2.6	0.2
3	202	0.9	21.6	1.8	2.0	2.4
Av.	219	1.3	8.0	1.1	1.7	3.1
Adrenal Extract Group			(3 cc.)	(5 cc.)	(5 cc.)	(5 cc.)
4	230	2.3	23.0	11.5	4.0	2.2
5	220	1.1	4.6	11.0	11.7	28.9
6	208	2.5	19.5	15.1	9.7	1.7
Av.	219	1.9	15.7	12.5	8.5	10.9
Adrenal Gland Emulsion			(4 cc.)	(4 cc.)	(4 cc.)	(4 cc.)
7	223	2.5	13.2	1.0	1.1	1.5
8	230		10.2	3.7	1.9	2.4
9	217	4.3	9.0	1.5	0.3	4.1
Av.	223	2.2	10.8	2.1	1.1	2.7
Experiment 3 (Young adult female rats)						
Control Group						
1	148		1.5	2.2	2.6	4.2
2	168		34.1	43.5	16.6	19.8
Av.	158		17.8	22.8	9.6	12.0
Adrenal Extract Group			(1 cc.)	(1 cc.)	(1 cc.)	(1 cc.)
3	160		24.0	19.1	21.0	12.3
4	163		32.0	42.2	31.1	4.2
Av.	161		28.0	30.6	26.1	8.2
Experiment 4 (Young adult male rats)						
Control Group						
1	200		2.5	2.9	3.6	2.5
2	194		4.3	2.4	1.6	1.5
Av.	197		3.4	2.6	2.6	2.0
Adrenal Extract Group			(1 cc.)	(1 cc.)	(1 cc.)	(1 cc.)
3	219		6.9	4.5	3.3	2.4
4	193		3.1	4.9	4.5	1.8
Av.	206		5.0	4.7	3.9	2.1

Experiment 5 (Young adult female rats, each given by stomach tube 200 mg. racemic sodium B-hydroxybutyrate per day)

Control Group					
1	177	166.0	182.0	207.0	200.0
2	161	122.0	132.0	152.0	147.0
Av.	169	141.0	157.0	179.5	173.5
Adrenal Extract Group		(1 cc.)	(1 cc.)	(1 cc.)	(1 cc.)
3	172	127.0	220.	239.0	209.0
4	161	136.0	216.0	247.0	248.0
Av.	167	131.5	218.0	243.0	228.5
Control Group not fed ketone body (see control group of Exp. 3)					
Av.	158	17.8	22.8	9.6	12.0

had been receiving our usual stock diet[5] and fasting commenced. Water was allowed *ad lib.* Whenever adrenal cortex extract was given to one group the controls received an equal amount of physiological saline. All injections were made subcutaneously. Urine was collected under paraffine oil and the total ketone body content determined by Van Slyke's method.[6]

That the adrenal cortex extract used in these experiments increased the fasting ketosis is obvious from a perusal of Table I. Large doses had a much more definite influence (Exp. 1 and 2) than small doses (Exp. 3 and 4). The fasting ketosis of female rats (Control groups of Exp. 1 and 3) is greater than that of male rats (Control groups of Exp. 2 and 4). Adrenal cortex extract increases the ketosis of fasting female rats (Exp. 1 and to a less extent the lower dose Exp. 3) more than that of fasting male rats (Exp. 2 and to a less degree the low dose Exp. 4). The sodium salt of racemic B-hydroxy-butyric acid is oxidized to some extent by fasting rats while the administration of adrenal cortex extract reduces this utilization so that it is negligible.

The ketogenic substance in the adrenal cortex extract may be the active hormone of this tissue or one of the group of miscellaneous substances from various sources which have the property of inducing or increasing a ketosis. We subscribe to the former view for a crude 25% emulsion of rabbit adrenals prepared by grinding them with a synthetic hydrated aluminum silicate powder ("Permutit"), which has the property of absorbing epinephrine, and injected at once, gave no increase in the degree of ketosis (Exp. 2). There is little reason to believe that this preparation contained an appreciable amount of cortical extract and in any case it did not increase the ketosis. Furthermore, some of the extract used in Experiment 1 stood in the laboratory for 6 months and then after refrigeration was overheated just before injection. It was then

[5] MacKay, L. L., and MacKay, E. M., *Am. J. Physiol.*, 1927, **83**, 179.

[6] Van Slyke, D. D., *J. Biol. Chem.*, 1917, **32**, 455.

inactive both in regard to its ketogenic activity and its ability to maintain the life of adrenalectomized rats.

Conclusions. An active adrenal cortex extract has been found to increase the ketosis of fasting female rats when administered in large doses. The ketosis of fasting male rats is only slightly increased. Adrenal cortex extract abolishes the partial oxidation of B-hydroxybutyric acid when fed to fasting rats.

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Effect of Allantoin Upon Fibroblasts from Cardiac Explants in Tissue Culture.

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It is difficult surgically and frequently impossible to eliminate all necrotic tissue and purulent materials from some deep wounds such as those of osteomyelitis.[1] To cope with this situation Baer[2] introduced the living maggot treatment which brought about early and complete healing of infections that had been resistant to other forms of accepted therapy. The beneficial results are possibly due to a combination of factors: (1) the maggots feeding upon and thus thoroughly removing all the diseased tissue; (2) the ingestion and physical removal of the microorganisms, with subsequent sterilization of the wound; (3) the proteolytic activity of the maggots' enzymes breaking down the discharge and slough of the wounds into their end products; (4) the maggots crawling about in the wound, causing sufficient irritation to stimulate rapid growth.[3] In addition to these possibilities, it was believed that the larvae secreted some substance which stimulated directly the healing process.

Following investigations of the secretion of maggots, a substance was found which had the property of stimulating healing in infected wounds.[4] It was identified as allantoin, the principal terminol product of purine metabolism in animals below man. It seemed evident that the secretion of this substance into the wounds contributed to the remarkable healing effects obtained by maggot therapy; how-

1 Meyers, J., and Czaja, L. M., *Illinois Med. J.*, 1931, **60**, 124.

2 Baer, William S., *J. Bone and Joint Surg.*, 1931, **13**, 438.

3 Buchman, Joseph, *Ann. Surg.*, 1934, **99**, 251.

4 Robinson, William, *J. Parasit.*, 1935, **21**, 354.