

increase in the arginase content of the livers of the treated rats was evident (see Table I). In a few

TABLE I
EFFECT OF ADRENOCORTICOTROPIC HORMONE (ACT H) AND OF
CORTIN ON LIVER ARGINASE OF HYPOPHY-
SECTOMIZED RATS*

Exp. No.	Treatment	Liver Arginase Content		
		per g Liver (Units)	per 100 g Rat (Units)	Increase Per cent.
1	ACT H treated controls	2,700 1,675	8,400 4,800	+ 75
2	ACT H treated controls	2,350 800	8,450 2,640	+ 220
3	ACT H treated controls	2,000 1,200	7,200 3,960	+ 82
4	Cortin treated controls	2,550 925	7,900 2,700	+ 193

* All fasted 21 to 24 hours preceding autopsy, after an injection period of 15 days. Treated rats in expts. 1 to 3 received 3.9 mg of the hormone preparation daily and three times during the 24 hour fast. Rats in expt. 4 received 0.5 cc of Upjohn's Adrenal Cortical Extract twice daily, with three injections of 1 cc during the 24 hour fast. The rats in expts. 1 to 3 were males, approximately 50 days old at operation and injected from the first day p.o. on. Those of expt. 4 were females, operated when approximately 30 days and injected after a postoperative period of 1 to 2 weeks.

The ACT H preparation used contained about 5 to 10 per cent. lactogenic hormone and less than 1 per cent. of other known hormones. The adrenal weights of the treated animals average 51 mg, those of the controls 14 mg.

groups, liver arginase was determined after treatment with the same dose of ACT H in combination with lactogenic hormone; also after the same cortin treatment, combined with growth hormone. In each case increases were found, but these were somewhat less pronounced than those brought about by ACT H or cortin alone. Studies of the effect of various other purified pituitary hormones and of thyroxin are in progress. As was noted by Lightbody,⁵ we find the hyperthyroid state, produced by high doses of thyroxin in normal rats, to be associated with a tendency to increases in liver arginase; physiological doses of thyroxin in hypophysectomized rats seem to have the opposite effect.

The striking increases in liver arginase produced by pituitary adrenocorticotrophic preparations, as well as by an adrenal cortical extract, are in good agreement with the theory that the adrenal cortex plays a predominant role in the hormonal control of gluconeogenesis. It has been established through the work of Long⁶ and others, that certain hormones of the

adrenal cortex enable fasting animals to maintain or even increase their carbohydrate stores, at the expense of body proteins; a similar action of ACT H has been demonstrated by Bennet⁷ and has since been amply confirmed by us. It is obvious that the action of these hormones in increasing liver arginase would favor gluconeogenesis.⁸

Studies are under way as to the effect on liver arginase of purified pituitary hormones under varied conditions in normal and hypophysectomized rats. The liver arginase increasing activity of various pure adrenocortical steroids should also be tested.

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THE EFFECT OF A PREPARATION OF AMINE OXIDASE ON EXPERI- MENTAL HYPERTENSION

THERE is evidence that deamination but not decarboxylation of certain amino acids is incomplete in kidneys deficient in their supply of oxygen.^{1,2} Since decarboxylation of amino acids leads in many instances to the formation of pressor amines, it was believed that this process might be responsible for some varieties of arterial hypertension. Because the enzyme tyrosinase lowers the blood pressure of hypertensive animals and human beings,^{3,4} a phenolic compound is probably concerned in the existence of this condition. It was desirable that other enzymes with known activity be employed in arterial hypertension in order that something further be learned regarding the nature of the pressor substance or substances.

A preparation of hog liver containing active amine oxidase, an enzyme specific for certain amines,⁵ was, therefore, given to animals. The intravenous injection of a small amount of this material consistently lowered the blood pressure of hypertensive rats, affecting that of normal ones to a less extent. When this preparation was mixed with a solution of angiotonin or tyramine the pressor response of these substances was abolished. Rats which had been injected

¹ L. L. Bennet, *Proc. Soc. Exp. Biol. and Med.*, 37: 50, 1937.

² Liver and muscle glycogen and blood sugar were determined in all rats included in the table; nitrogen excretion during the 24-hour fasting period was determined in experiment 1. While these results will be presented elsewhere, it should be stated that they indicated a definitely increased rate of gluconeogenesis in the treated animals.

³ P. Holtz, K. Credner and H. Walter, *Zeits. physiol. Chem.*, 262: 111, 1939.

⁴ R. A. Bing, *Am. Jour. Physiol.*, 132: 497, 1941.

⁵ H. A. Schroeder and M. K. Adams, *Jour. Exp. Med.*, 73, 531, 1941.

⁶ H. A. Schroeder, *SCIENCE*, 93: 116, 1941.

⁷ H. Blaschko, D. Richter and H. Schlossman, *Biochem. Jour.*, 31: 2187, 1937.

Seyler), 148: 264, 1925) and Takehara (H. Takehara, *Jour. Biochem. (Tokyo)*, 28: 309, 1938), using xanthidol for urea determinations. Since no activator was added to the crude liver extracts the determinations are regarded as indicating the amount of naturally activated arginase only. All rats were fasted 21 to 24 hours preceding autopsy.

⁵ D. H. Lightbody, E. Witt and A. Kleinman, *Proc. Soc. Exp. Biol. and Med.*, 46: 472, 1941.

⁶ C. N. H. Long, B. Katzin and E. G. Fry, *Endocrinology*, 26: 309, 1940.

with the enzyme were partially or totally refractory to the pressor action of renin.

The material (liver extract) was then injected intravenously in daily doses into five dogs made hypertensive by the Goldblatt⁶ technique. The blood pressure of all fell to normal levels within three to five days. The blood pressure of five normal dogs was similarly affected, but less. Associated with the fall was a reduction in the amount of urea nitrogen in the blood. A return to previous levels occurred a few days after the injections were stopped; when it was resumed the changes were repeated. In two hypertensive dogs the blood pressure and urea nitrogen both fell, but giving, subsequently, larger doses resulted in a further fall in blood pressure, a rapid rise in urea nitrogen, and death from uremia. Subcutaneous injections of this substance were followed by similar changes, but abscesses occurred. It was for that reason impossible to estimate the nature of the effect on hypertension. Larger quantities of the material

given by mouth had little or no effect in three dogs and one monkey. The changes observed did not result from the action of non-specific proteins; aliquot amounts of horse serum and ten preparations of inactivated enzyme, given daily, did not affect blood pressure.

The preparation appeared to be somewhat toxic to anesthetized rats, but did not adversely affect unanesthetized dogs. The material was insoluble and the enzyme unstable. It did not oxidize phenols. As many impurities were present it can not be concluded that the results were due to the action of amine oxidase, but this interpretation is possible. If so, amines are concerned in this type of hypertension.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE USE OF BROMOFORM IN THE SEPARATION OF NONCALCAREOUS MICROFOSSILS

POLLEN grains, plant spores, diatoms, sponge spicules and other microfossils are much more commonly present in unconsolidated sediments of Cenozoic age than has ordinarily been assumed. If properly identified, the microfauna and microflora of such deposits may prove to have great value in correlation and in studies dealing with the climatic and other conditions at the time and place of deposition. The separation of foraminifera and other calcareous microfossils from sediments by means of heavy liquids such as bromoform has become standard practice in many petrographical studies.¹ The value of such methods for the separation of the non-calcareous microfossils has not, however, been generally recognized.

During the last few years the writer² has been using a bromoform-acetone solution of a standard specific gravity to separate such fossils from various types of unconsolidated sediments ranging in age from Cretaceous to Recent. The technique that has been developed has led to the discovery of fossils in sediments that have formerly been considered to be completely barren. The method of separation and concentration is simple, rapid and complete. The microfossils of various types are concentrated together, thus making possible quantitative studies of the different elements

of the fauna and flora. The method is applicable to most types of microfossils found in sediments, including foraminifera, ostracods (with the two valves in place), radiolaria, silicoflagellates, siliceous sponge spicules, diatoms, pollen and spores of plants, and the light remains of plants and animals, which are often of diagnostic value. The method is especially valuable when dealing with sediments containing relatively few individuals.

The skeletal remains of most diatoms, radiolaria and silicisponges are composed of colloidal silica in the form of opal with a specific gravity of 1.9-2.3. Since quartz and the common clay minerals of which most sediments are composed have a somewhat higher density (2.5-2.7), it is possible to separate these siliceous fossils from unconsolidated sediments by means of a heavy liquid with a specific gravity of 2.3. Although the writer has found no previous reference to the use of heavy liquids in separating sponge spicules, radiolaria and silicoflagellates from sediments, he has found that the method has been occasionally used for the concentration of diatoms. Such a method is described by F. Hustedt.³ In this case Thoulet solution with a specific gravity of 2.3 was employed. This solution, however, is extremely poisonous and because of its corrosive properties and the difficulties involved in its use, it is not as suitable as bromoform.

Sediments also frequently contain small numbers of

⁶ H. Goldblatt, *Harvey Lectures*, 33: 237, 1937-1938.

¹ Marcus A. Hanna, *Econ. Geol.*, 22: 1, 14-17, 1927.

² Acknowledgment is gratefully expressed for financial aid received from Mr. Robert W. Sayles and the associates in science of Harvard University.

³ F. Hustedt, "Die Kieselalgen Deutschlands, Österreichs und der Schweiz" in Rabenhorst, L., *Kryptogamen-Flora von Deutschland, Österreich und der Schweiz*. Vol. vii, pp. 189-190, 1927.