B, or a physiologically similar substance produced when the adrenal cortex is stimulated by adrenocorticotropic extracts of the anterior lobe, causes increases in liver glycogen and blood sugar levels in these animals, only more slowly and incompletely restoring the muscle glycogen levels. When the cortical hormone and a glycostatic extract of the anterior lobe are administered simultaneously, the separate effects of each on liver and muscle are apparent.

10092

Sensitized Pupillary Dilator and Facial Muscles as Indicators of Sympathetic and Parasympathetic Substances in Blood.*

MORRIS B. BENDER. (Introduced by G. Shwartzman.)
From the Laboratories of the Mount Sinai Hospital, New York City.

It is well known that section of the cervical sympathetic renders the pupillary dilator fibers sensitive to circulating adrenaline. Therefore, any discharge of epinephrine into the blood stream would be indicated by greater dilatation in the denervated than in the normal pupil. The degree of dilatation varies with the concentration of adrenaline or sympathin in the blood and with the sensitivity of the radial fibers of the iris, which in turn depends on the completeness of denervation. This pupillary reaction may be used as an indicator for sympathetic hormonal activity.

An indicator for circulating parasympathetic substance (acetylcholine) may be prepared by sectioning the facial nerve (Bender[1]). This renders the denervated facial muscles sensitive to acetylcholine. In the presence of small amounts of acetylcholine, slow contraction in the paralyzed muscles may be seen. By repeated denervations the sensitivity of the reaction may be maintained for long periods.

On sectioning the superior cervical ganglion on one side and the facial nerve on the opposite, indicators for both sympathetic and parasympathetic substances were obtained. Monkeys, cats, dogs and rabbits were used. The operative procedures were simple, and the two sections were carried out in one period. Usually the left facial nerve and the right cervical sympathetic were cut.

The sensitization phenomenon appeared on the sympathectomized

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

1 Bender, M. B., *Am. J. Physiol.*, 1938, **121**, 609.

side 1 to 8 days after operation, and on the parasympathectomized side 4 to 14 days following the facial denervation. In the cat, dog, and rabbit, spontaneous tremors in the whiskers on the side of facial denervation appeared at the time the denervated facial muscles first became responsive to acetylcholine. Thus, by noting whether there were tremors in the whiskers, it was possible to predict when the denervated muscles were sensitive to acetylcholine.

Following this, the sensitization properties of these structures (pupil, nictitating membrane and face) continued to increase. The sensitivity was determined by repeated intravenous injections of adrenaline, acetylcholine with or without eserine, and combinations of these drugs. At the maximum point of sensitization it was found that (a) the minimal amount of adrenaline necessary to produce minimal dilatation of the denervated pupil was 0.25 gammas injected into the saphenous vein of a cat weighing 3.2 kg; (b) the minimal amount of acetylcholine necessary to produce a minimal contraction in the denervated facial muscles was 2.0 gammas injected into the saphenous vein of a monkey weighing 2.4 kg. In the eserinized animal, much lower concentrations (*e. g.,* 0.2 gammas) of acetylcholine were effective in producing facial contractions. The minimal dose in each species varied with the body weight. The determinations were made with the animals under nembutal or ether anesthesia. Cats were most sensitive indicators for sympathetic substances and monkeys acted best as indicators for circulating parasympathetic substances.

The minimal contractions were seen best in the ears and whiskers of the cat, dog and rabbit and in the eyelid and lips of the monkey. Any movement in these structures may be recorded photographically on a moving film.

The preparation was used for the following studies: (1) Under strong emotion, not only did the denervated pupil dilate, but the denervated facial muscles on the opposite side contracted, indicating that sympathetic and parasympathetic substances were discharged. In the cat, the pupillary dilatation was observed to be more conspicuous of the two, while in the monkey, the contraction in the face was the more obvious.

(2) Convulsions induced with metrazol produced dilatation of the denervated pupil which lasted for 5 and 20 minutes in the monkey and cat respectively. The effect, however, was more prominent in the cat. There was no manifest change in the denervated facial muscles.

(3) Injections of large amounts of insulin U 50-200 caused, in some monkeys, a slow and prolonged though not marked contraction

in the denervated face. This was not easily observed in other species.

(4) Injection of 4.0 gammas of acetylcholine into the general circulation of the cat resulted in a definite contraction in the denervated face within 7 seconds and dilatation of the denervated pupil in 17 seconds. The dilatation of the pupil must have been due to circulating adrenaline or sympathin. The latter supposition is explained by the fact that acetylcholine stimulated the adrenergic system (after 7 seconds—circulation time) liberating adrenaline into the blood stream which reached the denervated pupil 10 seconds later. The phenomenon of acetylcholine stimulating the formation of adrenaline was well illustrated in the cat.

TABLE I.

Cat weighing 2800 g. Left facial nerve and right superior cervical ganglion with sympathetic trunk were resected 10 days previously. Nembutal 1.4 cc was given intraperitoneally. Eserine 0.5 mg was given intramuscularly in order to inhibit cholinesterase activity in the cat used as the indicator. Atropine 1.0 mg was injected intramuscularly to counteract the muscarine effects of the injected acetylcholine. Two cc volumes of mixed acetylcholine and human blood serum were used. Several such mixtures were used in the determinations.

Serum concentration 25%.

Acetylcholine concentration 0.01% (1 cc equals 100.0 gammas).

10:45 A.M.—Threshold dose for minimal contraction—3.0 gammas into saphenous vein.

Aliquots injected into saphenous vein		Duration of serum mixed with Ach. in seconds	Results: Minimal contraction in ear	% hydrolyzed of 200 gammas
Vol/cc	Amt/gamma			
0.1	10	30	+	
0.1	10	40	±	
0.1	10	60	0	60%
0.2	20	60	++	
0.2	20	80	±	
0.2	20	110	0	
0.3	30	110	±	
0.3	30	180	0	92.5%
0.4	40	90	+	
0.4	40	120	+	
0.4	40	160	±	
0.5	50	160	+	
0.8	80	200	0	
1.0	100	220	±	96%
1.4	140	240	0	
1.6	160	240	0	
2.0	200	180	+	
2.0	200	255	±	98%

11:11 A.M.—Threshold dose: 4.0 gammas ± into saphenous vein.

Cerebrospinal fluid showed similar cholinesterase activity, but at a much slower rate.

The reverse of this, *i. e.*, adrenaline stimulating the secretion of acetylcholine was also found, but thus far only in 2 monkeys. Injections of large amounts of adrenaline, 400 to 1000 gammas, caused

a pronounced dilatation of the denervated pupil followed in 2 to 3 minutes by a slow contracture in the denervated face.

(5) The denervated face was also used as an end point in the determinations of rates of hydrolysis of acetylcholine by blood, and other body tissues and fluids. The great advantage of this preparation is that small (physiologic) amounts of acetylcholine could be used in these determinations and the indicator reaction is almost specific for acetylcholine.

Summary. (1) A preparation is described in which sympathomimetic and parasympathomimetic substances could be detected in the blood. (2) Any discharge of either or both of these substances under various physiologic states was indicated by dilatation of the denervated pupil or by contraction in the facial muscles respectively. (3) Under emotional stress, the cat exhibited predominantly sympathetic activity while the monkey showed parasympathetic activity in their denervated structures. (4) The denervated facial muscles were used as indicators of end points in the determinations of rates of hydrolysis by sera and cerebrospinal fluids.

10093 P

A Bacterial Enzyme Which Converts Creatine into its Anhydride Creatinine.*

RENÉ J. DUBOS AND BENJAMIN F. MILLER.

From the Hospital of the Rockefeller Institute for Medical Research, N. Y., and the Department of Medicine, University of Chicago.

We have previously described the preparation from two bacterial species (NC and HR) of enzymic systems that are highly specific for the decomposition of creatine and creatinine.[1, 2] These enzymic systems function only under aërobic conditions and convert creatine and creatinine into urea, ammonia, and other compounds. We have now observed that the washed cells of the NC bacterial species are capable of converting creatine into creatinine under anaërobic conditions; the creatinine so produced can then be decomposed by the same bacterial suspension when the conditions are rendered aërobic.

* This investigation was aided by a grant to one of the authors, B. F. Miller, from the John and Mary R. Markle Foundation, New York City.

1 Miller, B. F., and Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 335.

2 Dubos, R. J., and Miller, B. F., *J. Biol. Chem.*, 1937, **121**, 429.