the transaminase reaction and amino acid decarboxylation; blocking these reactions by a deficiency of B_6 would be expected to decrease the utilization of L-amino acids and ammonium citrate. A mechanism by which B_6 may affect the utilization of D-amino acids has not been demonstrated. The data indicate that the administration of D-amino acids to the B_6 deficient animal causes the excretion of a large amount of dietary nitrogen which is otherwise utilized. The mechanism by which the toxicity of D-serine, administered by stomach tube, is reduced by simultaneous administration of pyridoxine(9) may be related to this effect. Since the only demonstrated reaction for the metabolism of D-amino acids is through the action of D-amino acid oxidase, it is possible that a deficiency of B_6 decreases the utilization of D-amino acids by its effect upon this enzyme,

Summary. Homogenized kidney from rats deficient in vitamin B_6 exhibited only one third as much D-amino acid oxidase activity as did normal rat kidney homogenates. However, when the food intake of control animals was restricted to that of the B_6 deficient group there was no significant difference between the two groups in their kidney D-amino acid oxidase activity.

A gavage supplement of D-amino acids (leucine, isoleucine, phenylalanine and valine) to vitamin B_6 deficient rats greatly depresses dietary nitrogen utilization. Simultaneous injection of, or an adequate dietary supply of, vitamin B_6 completely overcomes this effect.

Supplements of the L-isomers of the same amino acids or an equivalent amount of inorganic nitrogen effected a smaller depression of dietary nitrogen utilization, thus pointing to a specific relationship between D-amino acid metabolism and vitamin B_6.

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Adrenal Function and Blood Electrolytes. (17612)

J. M. ROGOFF, JOSEPH M. QUASHNOCK, E. NOLA NIXON, AND ALAN W. ROSENBERG.

From the Laboratory of Experimental Endocrinology and the Department of Physiological Chemistry School of Medicine, University of Pittsburgh.*

Prevailing concepts attribute special physiological significance to the levels of the blood sodium and potassium in relation to steroid hormone function of the adrenal cortex. They are based on the observation that a reduction of sodium and increase of potassium occurs in the blood serum of adrenalectomized animals, and that administration of desoxycorticosterone or its acetate can effect a reversal of that change. It is maintained that this "corticoid" is a specific hormone of the adrenal cortex whose function is to regulate the balance between those electrolytes in the blood. The probability that loss of something other than any of the adrenal steroids might be responsible for the disturbed electrolyte levels in the blood of adrenalectomized animals has not been given serious attention. In view of the fact that adrenalectomy deprives an animal of function of the adrenal medulla as well as of alleged functions of cortical steroids, we undertook this investigation to determine if the physiological secretion of epinephrine might exercise an influence upon the blood sodium and potassium. A possible relationship between epinephrine action and serum potassium is suggested, also, from the results of various pharmacological investigations.

The demonstration by Rogoff and Stewart(1,2) that sodium chloride is diminished

* Supported by the G. N. Stewart Memorial Fund.

1. Rogoff, J. M., and Stewart, G. N., Demonstration of adrenalectomized animals, 38th annual meeting of the American Physiological Society, Cleveland, 1925.

in the blood of completely adrenalectomized dogs included the fact that at least 20% of the animals did not develop this change although other changes were present and the animals succumbed to the results of adrenal cortical insufficiency. Bauman and Kurland(3) obtained similar evidence and added the information that an elevation of plasma potassium occurred in adrenalectomized cats. Others have come to consider this as the pathognomonic evidence of adrenal cortical insufficiency although the idea does not have conclusive and unequivocal experimental support. In many instances the increase in potassium can be regarded as a concomitant of hemoconcentration. Significant evidence on this question is available from the electrical conductivity, specific gravity, and refractometric measurements that were reported by Rogoff and Stewart(1,2). The pharmacodynamic effects of adrenalin include an influence upon serum potassium. It seemed possible that the physiological secretion of epinephrine from the adrenal medulla might be concerned with regulation of potassium in the blood, perhaps more so than the influence of the supposed steroid hormone, desoxycorticosterone. This question lends itself to satisfactory quantitative study. The results of such an investigation are reported here.

There is agreement on the part of different investigators that administration of adrenalin does not result in a significant change in the level of blood sodium. The dosage employed in various studies upon human subjects as well as experimental laboratory animals was such as to induce hyperglycemia, glycosuria, elevation of blood pressure, and lymphocytosis, with or without a concomitant elevation in the level of blood serum potassium(4,5,6). However, conflicting results have been recorded in the literature concerning the influence of adrenalin upon serum potassium. Our experiments indicate that the discrepancies in the results of different investigators probably can be explained by the differences in dosage and method or rate of administration of the adrenalin. In this connection, is may be pointed out that dosage determines whether injected adrenalin produces only a glycemic response or both hyperglycemia and arterial hypertension. It has been reported that an increase in serum potassium is essential for production of a concomitant rise in blood pressure(7). Such an effect requires a much larger dose of adrenalin than is effective in producing a rise in blood sugar. It was found by McGuigan and Higgins(7) that the potassium ion, in certain dosage, is capable of eliciting responses which simulate sympathomimetic action of epinephrine. This observation, together with the fact that administration of adrenalin was found to increase the level of potassium in the blood serum, led Camp and Higgins(8) to conclude that the effects of adrenalin are, indeed, really due to the action of potassium. Camp(9) remarked that "The increase in serum potassium in Addison's disease would seem to be due to improper distribution of the potassium ion consequent to epinephrine deficiency."

Examination of the results reported by a number of investigators reveals that an elevation of the level of potassium followed administration of adrenalin in those cases where the dosage was relatively high, compared with the known range of physiological secretion of the hormone from the adrenals, or where smaller doses were introduced into the circulation at a rapid rate. In some instances the dosage and rate of injection of the adrenalin were such as yielded a preliminary, transient, rise in potassium which was succeeded by a more sustained decline lasting about one hour and then returning to the

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initial level(10,11,12).

In the observations made by Keys(11) on normal men, intravenous administration of adrenalin resulted in a decline in the level of potassium. In experimental laboratory animals, increase of serum potassium followed the injection of adrenalin. The different results can be accounted for by difference in dosage and rate of administration, *i.e.*, different amounts and concentrations of adrenalin in circulation. Brewer, Larson and Schroeder(12), in similar experiments, reported a preliminary rise followed by a fall in potassium, induced by adrenalin in both man and laboratory animals. Commenting on the difference between their results and those of Keys, they state, "In man as in the dog an interval of several minutes between injection of epinephrine and blood sampling allows sufficient time for the transient rise to have been replaced by a significant decrease in the blood potassium level."

It appears to us that the difference in results probably is due to the fact that in the experiments upon human subjects, by Keys, the effective dosage of adrenalin was in the physiological rather than in the pharmacological range. It was remarked by Brewer, *et al.* that "the possibility that a 'pure' fall in serum potassium might be produced if the proper dosage and injection rate for epinephrine could be found immediately presented itself." Obviously, this was accomplished in the experiments on men, by Keys.

Our experiments illustrate that the proper dosage and injection rate for eliciting a fall in the blood serum potassium is easily determined. If adrenalin is introduced into the blood stream in an amount and at a rate that corresponds to the physiological rate of epinephrine secretion by the adrenals, only a decline in potassium is produced in dogs. Other effects result from administration of doses that are above the physiological range. Such pharmacological reactions may result also from too rapid injection of smaller amounts of adrenalin.

Stewart and Rogoff(13) established that the average physiological rate of epinephrine secretion from the adrenals is between 0.0002 mg and 0.00025 mg per kg of body weight per minute. When administered at such a rate, our experiments indicate that adrenalin always causes a decline in the level of serum potassium. Therefore, we believe that this effect is a physiological reaction. Accordingly, it is highly probable that a quantitative influence upon the regulation of the level of blood potassium is exercised by the physiological epinephrine secretion from the adrenal medulla.

All of our experiments were performed on dogs. Preliminary studies were made to determine the normal range of blood serum sodium and potassium for dogs, under the ordinary conditions of our laboratory. The influence of physiological dosage of adrenalin upon the serum levels of these electrolytes was studied *a* in normal dogs, *b* in dogs with reduction or suppression of epinephrine secretion from the adrenals, *c* completely adrenalectomized animals, with and without administration of interrenalin (adrenal cortex extract).† All of the measurements of sodium and potassium were made by two of us (J.M.Q. and A.W.R.), employing the flame photometer; the operative and experimental procedures were performed by the other two (J.M.R. and E.N.N.). Adrenalin was administered by constant intravenous injection employing the apparatus described by Rogoff(14).

In nearly all of the experiments in which adrenalin was administered a blood specimen was obtained before the injection was begun, another after about 45 minutes of injection and a third about an hour after the injection was discontinued. In some of the experiments a specimen was also taken about 2 to 3 minutes after the beginning of the injection.

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† We are indebted to the Upjohn Company for a generous supply of adrenal cortex extract used in these experiments.

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TABLE I.

Effect of Constant Intravenous Injection of Physiological Quantities of Adrenalin upon Serum Sodium and Potassium in Normal Dogs and in Dogs After Reduction or Suppression of the Epinephrine Secretion from the Adrenal Glands. Dog, ♂.

		Adrenalin inj.					
		Before		During		After	
Date	Dose	Na	K	Na	K	Na	K
11/25/47	.14	153.8	5.27	144.6	5.27		
				152.4	4.60	160.9	3.59
28	.28	162.8	4.58	142.3	5.38		
				158.4	4.02	150.7	4.98
12/16	.28	148.9	4.76	145.2	5.04		
					4.47	154.8	4.87
19	.14	152.3	5.96	149.9	6.21		
				154.5	6.23	160.7	5.90
1/23/48				Right adrenal denervated			
26	.28	144.1	5.00	152.2	4.40		
				144.9	5.88	145.0	5.26
29	.14	172.9	5.65	169.1	4.97		
				178.1	5.21	163.7	5.47
2/ 6				Left adrenal excised			
9	.15	148.0	6.27	147.8	5.13		
				149.8	5.47	148.9	5.34
3/ 4	.72	152.1	5.87	161.4	4.17	154.0	5.13
30	.30	161.4	4.14	149.1	4.06	165.6	5.16
4/13				Right adrenal denervated further			
16	.30	109.3	5.36	107.7	5.73	117.3	4.66
20	.30	126.5	4.63	137.8	3.95	129.7	4.51
26	.15	154.8	4.90			150.1	3.72

The doses and rate of injection of adrenalin corresponded to amounts that were within the range which represents the minimum and maximum epinephrine output from the adrenal glands, under ordinary experimental conditions, *i.e.*, between approximately 0.0001 mg, and 0.001 mg per kg of body weight per minute. Seventy-one determinations on blood specimens obtained at intervals from 11 normal dogs yielded an average of 143 milliequivalents per liter for sodium. Sixty-nine determinations on the same animals and mostly on the corresponding blood specimens yielded an average of 5.1 milliequivalents per liter for potassium.

Double adrenalectomy was performed in 7 dogs. Two of these were treated with the adrenal cortical hormone, interrenalin. In 5 dogs, one adrenal was excised and the other denervated, an interval being allowed between operations for a series of blood studies. The remaining (denervated) adrenal was finally excised in one of these dogs, thus adding another to the group of double adrenalectomies. The remaining 4 animals were subjected to repeated denervation of the gland, later, to control the possibility of regeneration of the secretory nerves. These 4 dogs remained alive. Table I illustrates the results obtained in one of the dogs after suppression of epinephrine secretion. Table II presents experiments with 2 dogs, after bilateral adrenalectomy. In the 12 experimental animals, a total of 142 determinations of K and Na in the blood serum were made before the operations, 74 after excision or denervation of one adrenal, 138 after denervation or excision of the second adrenal, and 39 after repeated denervation or excision of the remaining gland. In all, 393 determinations were made in these experimental animals, in addition to the preliminary series that was performed on the normal, control dogs.

In general, the results of our experiments indicate that constant intravenous administration of adrenalin, in physiological dosage, does not influence the level of blood serum sodium. A small elevation that sometimes was observed could be accounted for by the salt added to the circulating blood in the isotonic

TABLE II.

Effect of Constant Intravenous Injection of Physiological Quantities of Adrenalin upon Blood Sodium and Potassium in Completely Adrenalectomized Dogs.

Dog	Date	Adrenalin injection: Dose	Before: Na	Before: K	During: Na	During: K	After: Na	After: K	Remarks
A	6/9/47	.13	112.6	7.52	124.6	5.54	126.0	5.36	2nd adrenal excised 6/6/47. Died a few hrs after adrenalin inj.
B	9	.20	137.0	5.16	133.4	3.70	138.2	3.98	2nd adrenal excised 6/6/47. Cortical extr. 6/7/47 to 7/5/47.
	7/8	.20	120.5	6.31	123.3	4.06	129.5	5.12	Extr. discontinued 3 days.
	11	.20	144.4	7.66	145.7	5.87	145.9	6.11	Extr. discontinued 6 days.

saline solution in which the adrenalin dilutions were prepared. This was of the same order of magnitude as was found when saline solution, without added adrenalin, was administered as a control experiment. On the other hand, diminution in the level of potassium nearly always (about 90% of cases) occurred. The results were the same in normal dogs, in those in which the adrenal epinephrine output was reduced or suppressed, and in bilaterally adrenalectomized animals in which elevation of potassium in the blood serum was present with or without diminution of the sodium level. While there were indications that the relation between epinephrine action and potassium level in the blood might be quantitative, it would require many more experiments to determine that point with certainty.

In completely adrenalectomized dogs, the increase in serum potassium often was associated with a decline in sodium. Frequently, the rise in potassium occurred without a change in the sodium and, occasionally, a fall in sodium level was found in the absence of a significant change in potassium. Alteration in the level of one or both of the electrolytes after adrenalectomy sometimes was found early but more often it developed and became more marked as the animal's condition became critical and onset of a moribund state was evident. In a significant number of experiments, there was a moderate rise in potassium, without change in sodium, for a day or two after unilateral adrenalectomy or adrenal denervation. In cases of bilateral excision of the glands, such a temporary rise in potassium level may occur after each operation. When the second adrenal is excised, however, that rise in potassium usually is followed by a more marked increase developing with the onset of the phenomena of acute adrenal cortical insufficiency. If the adrenal cortical hormone, interrenalin, is administered in proper dosage following excision of the second adrenal, the early temporary rise in potassium sometimes occurs and the more marked change is not seen until after cessation of the hormone treatment, when it develops usually about the same time as after second adrenalectomy in untreated animals.

These observations, together with the fact

that the increase in blood serum potassium can be prevented or corrected by administration of epinephrine in such amounts as are liberated normally from the adrenal glands, suggest the possibility or, indeed, the probability that any physiological role of the adrenal gland in the regulation of blood potassium is concerned with function of the epinephrine secretion from the adrenal medulla rather than the alleged function of a specific steroid hormone, actual secretion of which by the cortex has not been proven.

In some of our experimental animals one adrenal was denervated or excised and later the order of operation was reversed on the remaining gland. A third operation was performed, still later, to effect more marked reduction or total suppression of epinephrine secretion from the remaining gland (denervated). In a few experiments this gland was finally excised, placing the animal in the group of dogs with bilateral adrenalectomy. The results of experiments on constant intravenous injection of adrenalin, in amounts that correspond with the physiological epinephrine output from the adrenals, are illustrated in Table I. This experimental animal was selected for the table as a sample because it demonstrates, in one and the same animal, results obtained on the effect of adrenalin under normal conditions, after partial reduction of the animal's epinephrine secretion from the adrenals, and after operation for further reduction or suppression of the adrenal secretion.

The table illustrates that a reduction of blood serum potassium is effected by the action of physiological quantities of adrenalin. Amounts that are equivalent to the minimal rate of epinephrine secretion from the adrenals are effective in normal animals and in those that have been subjected to reduction or suppression of the epinephrine secretion. In completely adrenalectomized animals the same amounts of adrenalin are effective in diminishing the blood serum potassium regardless of whether it has or has not been elevated following loss of the adrenals.

An observation of some interest was made in a few of the experiments. It was found that in cases where there was a high potassium and low sodium, the adrenalin injections were followed by very toxic manifestations. In some cases it was necessary to institute treatment with cortical extract and intravenous saline infusions to save the animals. Early deaths occurred among such adrenalectomized animals if treatment was not resorted to soon enough. This observation is important in view of the undesirable, sometimes dangerous, reactions induced by adrenalin when administered to patients with Addison's disease.

Some of the results of experiments on 2 completely adrenalectomized animals in the series were selected for Table II, to illustrate the influence of physiological quantities of adrenalin in dogs deprived of both cortex and medulla of the adrenals. Both animals show the adrenalin effect to be the same as in normal dogs or in those in which only the epinephrine secretion from the medulla was interfered with. Dog "A" survived only a few hours after the adrenalin was administered, although he appeared to be in excellent condition at the beginning of the experiment. Dog "B" illustrates the lack of relationship between the levels of sodium and potassium when both are showing changes. In this animal life was prolonged by administration of interrenalin. One observation during that period is given and two observations that were made after hormone treatment was discontinued. The dog survived 10 days after cessation of treatment. In both tables, the dosage of adrenalin is given as μg per kg of body weight per minute. The amounts of Na and K are expressed in milliequivalents per liter of blood serum. Where two results are listed in the column under "During" adrenalin injection, the first represents a specimen obtained 2-3 minutes after beginning of the injection; the others were obtained about 45 minutes after beginning of the injection.

Inasmuch as reduction or suppression of epinephrine secretion from the adrenal glands causes not more than a moderate and transient elevation of blood potassium while complete adrenalectomy is generally followed by a persistent and more marked rise, the suggestion made previously, that the medulla rather than the cortex of the gland might be concerned

with regulation of blood potassium, may be considered debatable. However, it is possible that an influence upon regulation of potassium in the blood can be exercised by epinephrine secretion from the medulla, loss of which can be compensated for by the function of other mechanisms. It is conceivable that such a compensatory mechanism, or mechanisms, could be so affected by the profound metabolic disturbance which results from loss of cortical function that the ability of the organism to maintain a normal level of blood potassium is lost. We believe that the foregoing evidence, in support of the possibility that regulation of blood potassium may be accomplished by functional activity of the adrenal medulla, is at least as credible as that which attributes such a function to the cortex.

Summary. 1. The physiological role of the adrenal glands in relation to blood potassium, hitherto attributed to function of the cortex or one of its steroid components, can be ascribed to a function of epinephrine secretion from the medulla.

2. The level of potassium in the blood serum is diminished by intravenous administration of adrenalin in such amounts and rate of injection as correspond with the physiological rate of epinephrine secretion from the adrenal glands.

3. This effect is obtained in normal dogs and in animals with reduced or suppressed epinephrine secretion. The elevation of blood potassium, which occurs in adrenalectomized dogs, can be corrected by constant intravenous injection of physiological dosage of adrenalin.

4. Higher dosage may effect a rise in serum potassium. A moderate increase in dosage usually induces a preliminary rise followed by a more lasting reduction in the level of potassium. This can also result from too rapid injection of smaller doses.

5. Significant changes in sodium were not constant. The supposed dependence upon the adrenal cortex for maintenance of a physiological equilibrium between sodium and potassium is not supported by the results of our experiments.

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Effect of Cholesterol on Antigenicity of Streptolysin O.* (17613)

G. S. TURNER AND E. IRENE PENTZ. (Introduced by Alvin F. Coburn.)

From the Rheumatic Fever Research Institute, Northwestern University Medical School, Chicago, Ill.

The hemolysins contained in culture filtrates of hemolytic streptococci have provided abundant research material since their discovery by Marmorek(1). Despite initial controversy, the existence of 2 distinct varieties of hemolysin is now well established, Todd(2), Barnard and Todd(3), Herbert and Todd(4), Herbert and Todd(5). The oxygen labile streptolysin O has received much attention in the literature, and among other interesting properties is the neutralization of its hemolytic effects by suspensions of cholesterol in high dilution, Hewitt and Todd(6). Cohen and Schwachman(7) noted that pneu-

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