

COMPARISON OF INDUCED ELECTROLYTE DISTURBANCE IN NORMAL AND SENSITIZED GUINEA PIGS

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THE object of this work was to compare the response of sensitized and nonsensitized animals to factors which alter electrolyte equilibrium. The effects on tissue electrolyte content of two techniques which induce sodium retention and one which causes sodium depletion have been investigated.

METHODS

Groups of both sensitized and nonsensitized adult white guinea pigs (A.R.C. stock) with a body weight of 200 to 400 grams were subjected prior to death to one of three procedures which induce electrolyte disturbances. Groups of not less than five animals were used in each case.

Analysis of Muscles.—Animals were killed by a blow on the head. About 100 mg. of the tissue required was removed, wiped gently on filter paper to remove blood or mucus, and placed in a weighed glass tube which was then reweighed. Four muscles were studied—uterus, ileum, gastrocnemius, and diaphragm. The mucosa and endometrium of the ileum and uterus, respectively, were not removed. The muscles were dried for seventeen hours at 120° C. and weighed again one hour after removal from the oven. The tissues were dissolved in 1 ml. 50 per cent (V/V) nitric acid in a boiling water bath. This solution was diluted appropriately with distilled water for estimation of sodium and potassium by flame photometry. Duplicate estimations were always made.

Analysis of Serum.—Blood was withdrawn from the jugular vein after the animals had been stunned. Serum sodium and potassium were estimated by flame photometry after suitable dilution with distilled water.

Saturated Sodium Chloride Injection.—An injection of 0.2 ml. of saturated sodium chloride per 100 grams of body weight was given intraperitoneally a half-hour prior to death.

Administration of DOCA.—Daily sterile injections of 0.5 mg. (0.1 ml.) of DOCA in arachis oil were given intramuscularly to one of the four limbs in turn. Injections were continued for seven days, and the animals were killed twenty-four hours after the final injection. Control animals were treated with the hormone vehicle alone (0.1 ml.). With the sensitized animals, injections were arranged so that the animal would be killed twenty-one days after sensitization.

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Intraperitoneal Glucose Injection.—An injection of 10 ml. of 5 per cent glucose solution per 100 grams of body weight was given intraperitoneally three hours prior to death. At death the intraperitoneal fluid was withdrawn by suction and analyzed for sodium and potassium content by flame photometry.

Sensitization Methods.—Except for the DOCA-treated animals, guinea pigs were sensitized by subdermal injection of 0.75 ml. of 10 per cent crystalline egg albumin solution under sterile conditions. Animals were killed twenty-one days later.

The DOCA-treated animals were passively sensitized by injection of 0.5 ml. rabbit serum intravenously, the rabbit having previously been sensitized with egg albumin. For this, we used a modification of Slavin's⁵ technique, which involves using a calcium alginate gel as an antigen depot. An injection of 20 ml. of the following solution was given intraperitoneally to each rabbit:

5 Gm. crystalline egg albumin
5 Gm. sodium alginate
0.5 Gm. phenol
Distilled water to 100 ml.

This injection was followed immediately by 10 ml. of 2 per cent calcium chloride given into the same site. Three weeks later a booster dose of egg albumin was given intravenously, and after a further ten days blood was withdrawn for use.

A test for sensitization was made by adding a suitable dose of antigen to a bath containing Krebs solution in which was suspended a strip of ileum from the animal. The results from animals giving a negative response were excluded.

RESULTS

Effect of sensitization Alone on Composition of Serum and Muscles.—Figures given in Table I show that sensitization did not affect the water, sodium,

TABLE I

TISSUE OR FLUID	NO. OF ANIMALS	WATER (PER CENT)	SODIUM (mEq./L. OR mEq./100 GM. DRY TISSUE)	POTASSIUM (mEq./ L. OR mEq./100 GM. DRY TISSUE)
<i>Serum</i>				
Nonsensitized	18		129.0 ± 3.3	8.5 ± 0.27
Sensitized	16		131.0 ± 0.77	8.8 ± 0.26
<i>Uterus</i>				
Nonsensitized	8	79.8 ± 0.32	35.3 ± 0.77	38.0 ± 0.56
Sensitized	12	80.7 ± 0.15	33.8 ± 0.62	39.2 ± 0.64
<i>Ileum</i>				
Nonsensitized	8	79.4 ± 0.65	24.6 ± 0.71	50.7 ± 1.23
Sensitized	11	80.0 ± 0.26	23.7 ± 0.65	51.4 ± 0.51
<i>Gastrocnemius</i>				
Nonsensitized	9	P < 0.01 } 77.5 ± 0.15	11.4 ± 0.43	P < 0.001 } 49.0 ± 0.66
Sensitized	11	> 0.001 } 78.0 ± 0.09	10.6 ± 0.18	> 0.001 } 52.8 ± 0.56
<i>Diaphragm</i>				
Nonsensitized	9	P < 0.01 } 76.3 ± 0.34	14.2 ± 0.46	P < 0.001 } 42.2 ± 0.70
Sensitized	11	> 0.001 } 77.3 ± 0.12	14.1 ± 0.21	> 0.001 } 45.9 ± 0.36

The differences between the paired figures not bracketed can be accounted for by chance (P > 0.05).

or potassium content of serum or of smooth muscles. On the other hand, sensitization did appear to alter the total potassium content of striated muscles, significantly higher values being obtained in the sensitized group. In addition, the total water contents of the diaphragm and gastrocnemius were greater in the sensitized animals.

Effect of Administered Saline on Nonsensitized Animals.—Injection of saturated saline solution caused a significant uptake of sodium in all tissues investigated. Reference to Table II shows that serum sodium content rose

TABLE II. SODIUM CONTENT OF TISSUES (mEq./100 GM. DRY TISSUES AND mEq. PER LITER)

TISSUE	NORMAL	AFTER GLUCOSE	AFTER NaCl	AFTER DOCA	AFTER OIL
<i>Nonsensitized Animals</i>					
Uterus	35.3 ± 0.77	28.9 ± 0.57*	41.1 ± 1.10*	36.8 ± 0.63*	32.2 ± 0.61
Ileum	24.6 ± 0.71	22.1 ± 0.62*	30.9 ± 1.80*	26.1 ± 0.14*	20.9 ± 0.67
Gastrocnemius	11.4 ± 0.43	10.5 ± 0.55	14.7 ± 0.64*	13.0 ± 0.61*	9.2 ± 0.37
Diaphragm	14.2 ± 0.46	12.9 ± 0.41*	18.9 ± 0.70*	14.5 ± 0.71	13.0 ± 0.45
Serum	129.0 ± 3.3	133.0 ± 4.4	169.0 ± 2.6*	141.0 ± 3.1	134.0 ± 2.2
<i>Sensitized Animals</i>					
Uterus	33.8 ± 0.62	—	—	34.8 ± 0.57*†	36.8 ± 0.48
Ileum	23.7 ± 0.65	19.5 ± 0.40*†	29.9 ± 2.27*	22.7 ± 0.51†	22.3 ± 0.49
Gastrocnemius	10.6 ± 0.18	8.5 ± 0.24*†	10.9 ± 0.16†	11.1 ± 0.24†	10.7 ± 0.18
Diaphragm	14.1 ± 0.21	11.0 ± 0.37*†	15.2 ± 0.24*†	13.7 ± 0.34	13.4 ± 0.25
Serum	131.0 ± 0.8	125.0 ± 2.8*	145.0 ± 2.6*†	127.0 ± 1.5†	127.0 ± 0.70

*Significantly different from its own control ($P = < 0.05$) (for example, nonsensitized tissue after glucose treatment compared with normal nonsensitized value, or sensitized tissue after DOCA treatment compared with value of sensitized tissue after arachis oil treatment).

†Significantly different from corresponding nonsensitized tissue ($P = < 0.05$) (for example, value of sensitized tissue after NaCl treatment compared with nonsensitized tissue after NaCl treatment).

from 129 to 169 mEq. per liter a half-hour after the saline injection and that the sodium content of all the muscles was increased significantly. In addition the muscle potassium content was increased (Table III). There was no change, however, in total water content of the muscles (Table IV).

TABLE III. POTASSIUM CONTENT OF TISSUES (mEq./100 GM. DRY TISSUE OR mEq. PER LITER)

TISSUE	NORMAL	AFTER GLUCOSE	AFTER NaCl	AFTER DOCA	AFTER OIL
<i>Nonsensitized</i>					
Uterus	38.0 ± 0.56	42.7 ± 0.56*	42.5 ± 0.60*	39.5 ± 0.28	39.7 ± 0.48
Ileum	50.7 ± 1.23	51.4 ± 1.26	54.0 ± 0.78*	46.3 ± 0.81	47.2 ± 0.86
Gastrocnemius	49.0 ± 0.66	54.6 ± 0.71*	55.1 ± 0.67*	49.4 ± 0.32	48.5 ± 0.79
Diaphragm	42.2 ± 0.70	43.2 ± 0.81	46.9 ± 0.56*	42.5 ± 0.51	42.0 ± 0.45
Serum	8.5 ± 0.3	6.8 ± 0.9	7.4 ± 0.7	8.6 ± 0.4	8.6 ± 0.7
<i>Sensitized</i>					
Uterus	39.2 ± 0.64	—	—	41.6 ± 0.71†	40.4 ± 0.53
Ileum	51.4 ± 0.51	48.2 ± 0.40*†	48.6 ± 1.27*†	52.3 ± 0.84†	50.8 ± 0.53
Gastrocnemius	52.8 ± 0.56	52.0 ± 0.57†	51.5 ± 0.60†	50.8 ± 0.53†	50.3 ± 0.51
Diaphragm	45.9 ± 0.36	44.1 ± 0.37*	43.8 ± 0.43*†	44.8 ± 0.47†	45.0 ± 0.48
Serum	8.8 ± 0.3	9.1 ± 0.7	7.9 ± 0.4	8.7 ± 0.3	8.5 ± 0.4

*Significantly different from its own control ($P = < 0.05$) (for example, nonsensitized tissue after glucose treatment compared with normal nonsensitized value).

†Significantly different from corresponding nonsensitized tissue ($P = < 0.05$) (for example, value of sensitized tissue after NaCl treatment compared with nonsensitized tissue after NaCl treatment).

Effect of Saturated Saline Injection on Sensitized Animals.—The serum sodium level of sensitized animals was raised from 131 mEq. per liter to 145 mEq. per liter a half-hour after the saline injection (Table II). This increase

of only 14 mEq. per liter was in striking contrast to the elevation of 40 mEq. per liter which had been found in nonsensitized animals. This difference in response cannot be accounted for by chance ($P < 0.001$). Following the same pattern, the concentration of sodium within the muscles of sensitized animals was considerably less than that which had occurred in nonsensitized animals. Furthermore, the uptake of potassium by the muscles was less in the sensitized group (Table III) and the two striated muscles showed a lower water content (Table IV).

TABLE IV. PERCENTAGE WATER CONTENT OF TISSUES

TISSUE	NORMAL	AFTER GLUCOSE	AFTER NaCl	AFTER DOCA	AFTER OIL
<i>Nonsensitized</i>					
Uterus	79.9 ± 0.32	79.5 ± 0.31	79.0 ± 0.27	79.9 ± 0.02	79.8 ± 0.29
Ileum	79.4 ± 0.65	78.6 ± 0.29	78.9 ± 0.32	78.0 ± 0.02	77.8 ± 0.40
Gastrocnemius	77.5 ± 0.15	78.0 ± 0.21	77.5 ± 0.22	76.8 ± 0.02	76.5 ± 0.02
Diaphragm	76.3 ± 0.34	76.5 ± 0.41	76.6 ± 0.15	74.9 ± 0.02	75.2 ± 0.02
<i>Sensitized</i>					
Uterus	80.7 ± 0.15	—	—	81.0 ± 0.34†	81.4 ± 0.12
Ileum	80.0 ± 0.26	79.5 ± 0.19†	79.6 ± 0.28	79.8 ± 0.23†	79.4 ± 0.24
Gastrocnemius	78.0 ± 0.09	78.1 ± 0.16	76.3 ± 0.13*†	77.6 ± 0.22†	77.6 ± 0.14
Diaphragm	77.3 ± 0.12	77.1 ± 0.15	75.8 ± 0.16*†	76.8 ± 0.25†	76.8 ± 0.21

*Significantly different from its own control ($P = < 0.05$).

†Significantly different from corresponding nonsensitized tissue ($P = < 0.05$) (for example, value of sensitized tissue after DOCA compared with nonsensitized tissue after DOCA treatment).

From preliminary studies, it appeared that in sensitized animals the peak of sodium elevation in the tissues occurred one quarter of an hour after the saline injection. This peak was maintained at a similar level at one-half hour and one hour. The nonsensitized animals, with their greater response, required one-half hour to attain maximum sodium uptake in the tissues. There was a slight falling-off of effect after one hour. These results show that using a time interval of one-half hour in the main experiments was justified, since the maximum response in both groups was evidenced at this point.

Effect of DOCA Administration on Nonsensitized Animals.—DOCA treatment (0.5 mg. DOCA in arachis oil daily for seven days) caused a slight increase in serum sodium level from 134 mEq. per liter to 141 mEq. per liter. In three out of the four muscles investigated there was a highly significant increase in sodium content (P values of < 0.001 , $< 0.01 > 0.001$, and < 0.001). The hormone vehicle, arachis oil, itself had some effect in causing a fall in muscle sodium content.

DOCA administration did not affect the potassium or water content of the muscles significantly.

Effect of DOCA Administration on Sensitized Animals.—There was little appreciable response of sensitized animals to DOCA administration. The serum sodium level remained constant, and only one out of the four muscles investigated showed a significant increase in sodium ($P < 0.02 > 0.01$). DOCA had no effect on the potassium or water content of the muscles.

Thus, once again there was a striking difference in the responses of sensitized and nonsensitized animals. Statistical analysis of the results shows that the sodium content of both serum and muscles was significantly less in the sensitized group as compared with the nonsensitized group. Sensitized animals appeared to have a higher muscle potassium content, but this is partly attributable, in the gastrocnemius and the diaphragm, to the higher initial content resulting from sensitization alone. The sensitized group also showed a significantly higher muscle water content (Tables II, III, and IV).

Effect of Glucose Injection on Nonsensitized Animals.—It was found that three hours after glucose administration the muscles had become depleted in sodium. There was a net flux of sodium into the peritoneal fluid, such that its concentration approached that of serum. Figures given in Table II show that the total sodium content of each of the muscles investigated had fallen appreciably but serum sodium was not significantly changed. The concentration of sodium in the withdrawn fluid was 96.5 mEq. per liter, showing an influx of sodium into the peritoneal cavity of 9.3 mEq. per kilogram of body weight. The volume of fluid withdrawn was the same as that which had been injected (22.4 and 22.3 ml.). There was no change in the percentage water content of the muscles (Table IV).

The concentration of potassium in the intraperitoneal fluid was 2.92 mEq. per liter, thus showing an influx of 0.27 mEq. potassium into the peritoneal cavity per kilogram of body weight. Serum concentration showed no significant change, but there was some tendency for the muscles to take up potassium; two out of the four muscles showed a significant increase. This was possibly a compensatory increase toward balancing the sodium depletion.

Effect of Glucose Injection on Sensitized Animals.—The response of sensitized animals to this treatment was qualitatively similar to that of nonsensitized animals in so far as the muscles lost sodium. This effect was considerably exaggerated in sensitized animals, however; for instance the sodium content of diaphragm fell by 15 per cent (14.1 to 11.0 mEq. per 100 Gm. dry tissue), whereas that of nonsensitized animals fell by only 9 per cent (14.2 to 12.9 mEq. per 100 Gm. dry tissue). None of the differences in muscle sodium contents of the sensitized and nonsensitized animals could be accounted for by chance.

A further difference between the two groups was that muscles of sensitized animals tended to lose potassium, whereas those of nonsensitized animals showed an increase. Hence, the sensitized group showed a far greater electrolyte depletion than did the nonsensitized, the sodium loss was greater, and the compensatory potassium increase was not in evidence. In both groups, however, despite considerable change in sodium content, the total water content of the muscles remained essentially unchanged.

As with nonsensitized animals, glucose treatment did not significantly affect the sodium or potassium content of serum.

Analysis of the withdrawn intraperitoneal fluid revealed a significantly greater influx of potassium into the cavity in sensitized animals (0.35 mEq. per kilogram of body weight compared with 0.29 for normal animals, $P = 0.05$).

Sensitized animals also appeared to show a slightly greater influx of sodium (10.6 mEq. per kilogram of body weight compared with 9.3 for normal animals), but this difference can be accounted for by chance. In sensitized animals the amount of fluid recovered was slightly greater than that injected.

DISCUSSION

Effect of Sensitization on Tissue Sodium and Potassium Content.—The results have shown that sensitization alone caused no disturbance in the sodium or potassium content of either serum or smooth muscles. Surprisingly enough, striated muscles of sensitized guinea pigs were found to have higher potassium contents than those of normal nonsensitized animals. Both diaphragm and gastrocnemius showed significantly higher potassium and water concentrations but no significant change in sodium content. These results have been shown to be repeatable.

Some previous workers have reported electrolyte changes occurring in tissues during sensitization. Einbinder, Nelson, and Fox¹ reported that the main electrolyte shifts occurring in mice are that potassium and sodium are deposited in the bones. They reported a fall in sodium content of "viscera" (pooled heart, liver, spleen, and lungs). In mice there appears to be no uptake of potassium by skeletal muscle as was found in the present work on guinea pigs. This is possibly due to species variations, which is further evidenced by the fact that the *normal* potassium content of skeletal muscles in these two species is grossly dissimilar (393 mEq. per kilogram of wet tissue for mouse compared with 490 for guinea pig).

Further species differences in the response to sensitization are indicated in analysis of blood. Wenner and Buhrmester⁶ reported a striking elevation of serum potassium in rabbits, whereas in guinea pigs no change occurs. Nevertheless, sensitization is associated with some electrolyte disturbances in all three species on which reports are available.

Comparison of Nonsensitized With Sensitized Animals' Response to Induced Electrolyte Disturbance.—It has been shown that sensitized animals responded to a *lesser* extent than did nonsensitized animals to factors causing an increase in body sodium (DOCA or saline treatment), the tissue sodium content being elevated to a lesser extent. On the other hand, sensitized animals showed a *greater* response to sodium depletion (induced by injecting glucose solution), that is, the tissue sodium level fell to a greater extent.

The results have shown that muscles of sensitized animals lose sodium more readily than those of nonsensitized animals when electrolyte depletion is induced. On the other hand, they take up less sodium when the total body sodium is increased. Two of the techniques used involved intraperitoneal injection of a solution which served to alter the composition of the blood and surrounding tissues. It is possible, although not proved, that the passage of water and electrolytes into and out of the peritoneal cavity proceeds at different rates in the sensitized and nonsensitized animals.

DOCA treatment was ineffective in raising tissue sodium level in sensitized animals, although the dose used did cause sodium retention in nonsensitized guinea pigs. A possible explanation may be that sensitized animals develop refractoriness to DOCA more readily than do normal animals. It has been shown previously that a completely refractory state can be induced in animals with repeated injection of sodium-retaining hormones, including DOCA.²⁻⁴ For instance, using a "DOC beef adrenal protein mixture," these workers demonstrated complete refractoriness appearing as early as the fourth injection. Further evidence is required, however, to establish whether or not a shorter period of DOCA treatment than that used in the present experiments can cause sodium retention in sensitized animals and, hence, whether or not this refractoriness plays a significant role.

Another factor which may account for the differences found between sensitized and nonsensitized animals is that the mechanism controlling the intracellular concentration of electrolytes in the muscle is disturbed in sensitized animals. The high total potassium content found in striated muscles of untreated sensitized animals is possibly due to a high intracellular concentration, since serum level (and thus, presumably, extracellular concentration) remained normal. Since intracellular potassium concentration is so much greater than extracellular potassium, fluctuation in intracellular content would greatly influence the results of total muscle potassium.

Alternatively, the control of renal excretion of electrolytes may be disturbed in sensitized animals. For instance, if there were a relative lack of sodium-retaining hormones in sensitized animals, then a sodium load in the body would be lost more readily than normal. Hence, the blood and muscle sodium concentration would be lower in such animals. Conversely, if the body were depleted of sodium, then these animals could less readily correct this by retaining more sodium. This is in accordance with the results reported. In addition, some disturbance in factors controlling electrolyte balance could account for the high muscle potassium concentration found in two muscles of untreated sensitized animals.

SUMMARY

1. Estimations of water, sodium, and potassium content of uterus, ileum, gastrocnemius, diaphragm, and serum have been made on nonsensitized and actively sensitized guinea pigs. Sensitized animals were killed twenty-one days after antigen injection. It was found that neither the smooth muscles nor serum showed any differences in the two groups but the striated muscles of sensitized animals had an abnormally high potassium content.

2. Groups of normal and either actively or passively sensitized animals were subjected to electrolyte disturbance prior to death. Tissue analyses revealed that increasing the tissue sodium content (by intraperitoneal injection of saturated saline solution, 0.2 ml. per 100 grams of body weight, a half-hour prior to death or by seven daily injections of 0.5 mg. DOCA) caused a lesser uptake of sodium in sensitized animals than in normal animals.

3. On the other hand, the response of sensitized animals to a depletion of total body sodium (by intraperitoneal injection of 10 ml. of 5 per cent glucose solution per 100 grams of body weight three hours prior to death) was greater than normal.

4. Some factors which may contribute to an explanation of these results are discussed.

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