11956

Maintenance of Adrenalectomized Guinea Pigs.*

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Schachter and Bebee[1] have described a technic for adrenalectomizing guinea pigs, and have found this animal extremely sensitive to the absence of the adrenal cortex and very responsive to cortical extract, dying of adrenal insufficiency in 3 to 4 days after maintenance for 10 to 12 days on cortical extract.

During some other investigations on adrenalectomized guinea pigs, a few observations were made on the dietary requirements of normal and adrenalectomized guinea pigs, responsiveness to cortical hormone and salt therapy, and survival, which were thought worthwhile to report here.

The technic of adrenalectomy was somewhat similar to that of Schachter and Bebee, being performed in 2 stages. The right adrenal was removed first, 2 weeks elapsing before the left was removed. From experiments scattered from 1938 through 1940, over 40 animals were adrenalectomized. In 20 guinea pigs, the average survival after removal from hormone or salt therapy was 4.6 days average (extremes 2 to 10, depending upon age).

In some preliminary work on dietary requirements, several different rations were examined. These are listed in Table I.

The animals were individually caged and fed and watered daily from glass finger bowls.

Ration No. 1. Nine male guinea pigs, average body weight 612 g, were fed ration 1 and tap water. In 6 days, the smallest animal of the group had a swollen abdomen and some alopecia. In 10 days, one animal developed diarrhea and died on the 12th day. Two others were very weak on the 12th day. The average weight loss of the group was 57 g in this time. The calculated ascorbic acid intake was 20 to 40 mg daily per guinea pig.

Ration No. 2. Seven males, average body weight 680 g, were fed ration 2 and tap water for 30 days, at which time the average weight loss was 18 g, the losses being much greater for younger animals.

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1 Schachter, R. J., and Bebee, M. O., Jr., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 541.

TABLE I.
Experimental Diets for Guinea Pigs.

Item	Ration No.				
	1	2	3	4	5
Dextrinized starch (Corn Products)	—	—	55	—	50
Dextrose (Corn sugar)	55	55	—	56	—
Casein	20	20	18	18	20
"Celluflour"	10	10	—	—	—
Lard	—	—	—	—	7
Soybean oil (Archer-Daniel)	8	8	8	7	—
Corn oil ("Mazola")	—	—	—	—	9
Wheatgerm oil (cold pressed)	2	2	2	2	—
Salt mixture (1)	2	2	2	2	2
Yeast concentrate (2)	3	3	3	3	—
Dried brewer's yeast (Fleischman)	—	—	—	—	10
Ascorbic acid (3)	0.1	0.1	0.2	0.2	—
Oleum percomorphum (Mead-Johnson)	0.001	0.001	—	—	—
Codliver oil (USP)	—	—	2	2	2
Leaf-ground alfalfa (fresh dried)	—	10%*	—	—	—
Dehydrated, pulverized grass (4)	—	—	10	10	20%*

(1) Hubbell, Mendel, and Wakeman.[2]
(2) Upjohn. Dry powder concentrate.
(3) Cryst. Supplied by Chas. Pfizer and Co., Inc., New York.
(4) Canned under nitrogen. Supplied by Cerophyll Laboratories, Kansas City, Mo.
*Signifies that the item comprised 10% and 20% of rations 2 and 5 respectively.

Ration No. 3. Twelve males, average body weight 562 g, were fed ration 3 for 9 days. After 8 days, 4 animals were near collapse, and the average weight loss was 113 g. On the 9th day, 2 died in convulsions.

Ration No. 4. Ten males, average body weight 510 g, were fed this diet for 30 days. Two animals refused to eat and were discarded. One animal developed pneumonia, lost weight, and was discarded. Daily food consumption averaged 30 to 50 g. The group gained an average of 118 g in the 30 days. Hence this diet, containing 10% dehydrated grass, was fairly adequate for this period of time.

Ration No. 5. This ration has been tried on several animals at various times, and found to be somewhat superior to ration 4 for animals of all ages. As an example, 5 animals, average body weight 800 g, gained 85 to 130 g in 40 days on ration 5, containing 20% dehydrated grass.

These observations are in accord with those of Cannon and Emerson,[3] Kohler, Randle, Elvehjem, and Hart,[4] and Kohler, Randle,

[2] Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition*, 1937, **14**, 273.

[3] Cannon, M. D., and Emerson, G. A., *J. Nutrition*, 1939, **18**, 155.

[4] Kohler, G. O., Randle, S. B., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 154.

and Wagner[5] (*cf.* also Kohler and Schnabe[6]) which show that guinea pigs require a water-soluble factor obtained from grass juice in addition to the known B-complex and ascorbic acid, dying in 3 to 10 weeks if it is not supplied.

Fourteen guinea pigs, average body weight 498 g (420-585), were adrenalectomized in 2 stages, as described above. After removal of the first adrenal, they were fed ration 4. Five died in 19-30 days, with symptoms of a respiratory infection. All the others steadily gained weight for 30 days, when the second adrenal was removed. Seven unilaterally adrenalectomized guinea pigs served as controls. Desoxycorticosterone acetate in oil† in 0.5 to 1.0 mg doses was administered subcutaneously daily to the doubly adrenalectomized animals. Nine or 10 days after the second adrenal was removed, the average body weight of all animals, including the controls began to fall, and after 10 more days, had decreased 75 to 100 g. It is possible that the grass juice factor content of the grass powder had decreased in this time, as it had been kept in bottles in the refrigerator for some time. At this point, 10% freshly opened dehydrated grass was mixed with 90% ration 4, thus increasing the level of the grass juice factor. The controls gained 25 to 85 g in 6 days, whereas the doubly adrenalectomized animals continued to lose (25 to 70 g) in the same period of time. These results indicated that a diet adequate for normal guinea pigs and containing an excess of the grass juice factor, still failed to maintain adrenalectomized guinea pigs. The grass powder contained 4.65% K and 0.57% Na. Ration 4 contained 0.80% K, thus with 90% ration 4 and 10% additional grass powder, the K content increased to 1.2%, a 0.4% increase. It is doubtful that this much increase would be very detrimental when desoxycorticosterone acetate was being administered.

After this period, all animals were fed Purina "complete rabbit chow", whole oats, and fresh greens (lettuce and cabbage) daily, with the hormone therapy continued as before. On this high potassium diet, the doubly adrenalectomized animals gained an average of 145 g over a 26-day period, as compared with 40 g in the controls. Two animals in both groups died during this time, with symptoms of pneumonia, and showed heavy infiltration of macrophages in spleen imprints. Hormone therapy was then withdrawn from the remaining 7 animals in the doubly adrenalectomized group

[5] Kohler, G. O., Randle, S. B., and Wagner, J. R., *J. Biol. Chem.*, 1939, **128**, lv.

[6] Kohler, G. O., and Schnabe, C. F., Am. Chem. Soc., 99th Meeting, 1940.

† Supplied by Roche-Organon, Inc.

to test for possible hypertrophy of adrenal fragments or accessory bodies. In 5 days, 3 animals fell sharply in weight, the other 4 larger animals maintaining weight for 7 to 8 days before decreasing. One mg of desoxycorticosterone acetate prevented further weight loss. Hormone therapy was again discontinued, and the tap water was replaced by 1% NaCl, 0.2% $NaHCO_3$ drinking fluid. The 7 experimental guinea pigs increased in weight from an average of 629 to 758 g, a gain of 129 g in 41 days, as compared with an increase of 159 g in the controls. Thus salt intake replacing the desoxycorticosterone acetate acted for at least 6 weeks as a complete replacement therapy on the ration used.

To further test for hypertrophy of possible adrenal fragments or accessory bodies, the salt solution was then again replaced by tap water. Four of the 7 animals lost an average of 25 g in 6 days, while the larger animal maintained weight during this time. Salt was then resupplied, and 0.5 cc Wilson cortical extract administered daily for 3 days. The animals were fed ration 5. Two of them did not recover the weight loss suffered during the salt removal test, and died in 6 and 7 days. The remaining 5 guinea pigs gained an average of 107 g after 34 days on the new regime, and when salt solution was again replaced with tap water, they promptly died, surviving 4.6 days. They had lost an average of 92 g in this time. These 5 animals had been maintained on various dietary regimes and on salt and hormone therapies for over 5 months following adrenalectomy. In all probability, since these animals grew at a normal rate when compared with the control animals for a 40-day period in which they received salt drinking fluid but no cortical hormone, they might have been maintained for weeks at a time by such methods. Also, since 0.5-1.0 mg desoxycorticosterone acetate daily maintained adrenalectomized guinea pigs weighing 600 to 1000 g in a normal condition, as compared with 1.0 mg which is required by the immature rat,[7] and since the animals do not necessitate the use of low potassium-high sodium diets like adrenalectomized dogs, the guinea pig offers an extremely sensitive and inexpensive animal for investigations involving the adrenal cortex.

Summary. Several diets were tested for adequacy in the nutrition of the guinea pig, and the necessity of the grass juice factor for this animal was confirmed. A diet high in the grass juice factor and adequate for normal guinea pigs, failed to maintain adrenalectomized guinea pigs given amounts of desoxycorticosterone acetate which were adequate when a normal diet was fed. Adrenalectomized guinea

[7] Grollman, A., *J. Pharm. and Exp. Therap.*, 1939, **67**, 257.

pigs can be maintained for at least 6 weeks on a normal diet if salt drinking fluid is offered.

Desoxycorticosterone acetate subcutaneously administered in daily doses of 0.5 to 1.0 mg produces normal growth in adrenalectomized guinea pigs.

Even though adrenalectomized guinea pigs had been maintained for over 5 months by salt and hormone therapy, they died on an average of 4.6 days after discontinuing this therapy.

11957

Effect of Obstructive Jaundice on Polyarthritis in Rats.*

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Hench[1] reported that marked and generally complete remissions from various rheumatic symptoms occur in patients with the onset of spontaneous jaundice. Later[2] he reported further confirmatory observations and stressed the fact that the phenomenon seemed to depend upon the severity of jaundice as measured by the concentration of serum bilirubin rather than upon the type of jaundice.

Because of these clinical observations it seemed of interest to investigate the effect of obstructive jaundice on arthritis in an experimental animal. Collier[3] first described a spontaneous polyarthritis in rats. Findlay, Mackenzie, MacCallum and Klieneberger[4] reported a somewhat similar disease in rats and were able to cultivate a pleuropneumonia-like organism from affected joints which, upon inoculation into rats, reproduced the disease. A culture of Findlay's organism, designated L_7, was obtained.

Methods. Young albino rats (average age, 6 weeks; weight, 100 g) were used. For culturing the L_7 organisms 20% horse serum nutrient broth was employed, 48-hour cultures showing distinct turbidity or growth. The size of the infective dose of

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1 Hench, P. S., *Proc. Staff Meet., Mayo Clinic*, 1933, **8**, 430.

2 Hench, P. S., *Arch. Int. Med.*, 1938, **61**, 451.

3 Collier, W. A., *Geneesk. Tijdschr. Ned.-Ind.*, 1938, **78**, 2845.

4 Findlay, G. M., Mackenzie, R. D., MacCallum, F. O., and Klieneberger, E., *Lancet*, 1939, **2**, 7.