Failure of Liver Feeding to Counteract Cortisone Effects Other than Growth Inhibition. (20972)

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Ershoff(1) reported that addition of 10% whole liver powder to an adequate synthetic diet minimized certain effects of oral cortisone acetate upon the immature rat. These effects included retardation of growth, alopecia and death. The present authors(2) reported an additional effect of cortisone acetate in rats, *viz.*, hypertension. The experiments herein described sought to determine whether addition of liver powder to the diet might also prevent development of hypertension as well as of other effects characteristic of prolonged cortisone administration.

Methods. Sixty-four male Sprague-Dawley rats were purchased from the Holtzman-Rolfsmeyer Company at 4 weeks of age. After a 2 week period of acclimatization the animals were divided into 8 groups (Table I). The animals in the first 4 groups were unoperated; bilateral adrenalectomies were performed at the outset of the experimental period upon rats in the other 4 groups. All animals were given a sodium-restricted synthetic diet of the following composition: Dextrose 60%, Labco casein 24%, Wesson oil 10%, cod liver oil 2%, sodium chloride-free USP salt mixture No. 2, 3.3%, and ammonium chloride 0.67%. This was supplemented with 10 g of the following vitamin mixture per kilo of diet: Vit. B_1 100 mg, Vit. B_2 200 mg, Vit. B_6 100 mg, pantothenic acid 1 g, nicotinic acid 1 g, inositol 500 mg, choline 10 g, PABA 3 g, biotin 5 mg, folic acid 20 mg, Vit. E 1420 mg, Vit. K 1420 mg, and Vit. B_{12} triturate 1 g. This basic diet was adjusted to contain 10% desiccated whole liver* for the rats in the 2nd, 4th, 6th and 8th groups. Cortisone acetate† was administered to all groups. In Groups III, IV, VII and VIII 3 mg of the steroid was injected subcutaneously daily in order to ensure both the control and liver supplemented groups receiving equal amounts despite possible variations in food intake. In Groups I, II, V, VI,

TABLE I. Effect of Dietary Liver Supplementation on Action of Cortisone Acetate upon Growth, Blood Pressure, Serum Sodium, Sugar and Liver Glycogen Levels.

Group:	I	II	III	IV	V	VI	VII	VIII
Adrenals:	intact				excised			
Cortisone acetate, mg/day	±6*	±6*	3	3	±6*	±6*	3	3
Route of steroid admin.	per os		subcut.		per os		subcut.	
Liver in diet, %†	0	10	0	10	0	10	0	10
Initial wt, g	118	118	118	118	118	118	118	118
Range	105–131	106–131	104–130	106–128	102–133	102–134	100–132	104–134
Final wt, g	123	155	118	112	103	109	106	104
Range	97–142	137–181	110–131	100–125	90–120	92–128	85–115	92–118
Change in 14 days	+5	+37	0	−6	−15	−9	−12	−14
Initial blood pressure, mm Hg	112	110	115	105	115	117	113	112
Final blood pressure, mm Hg	170	173	171	169	189	174	179	173
Terminal serum Na,‡ mEq/L	148.	143.8	140.5	141.5	130.0	132.0	142.0	141.5
Terminal serum sugar,‡ mg %	99	215	285	283	95	132	270	130
Liver glycogen,§ mg/g	7.6	10.7	46.0	48.0	7.9	8.5	49.5	52.0

* Steroid incorporated in food conc. of 6 mg/10 g of diet. † Armour's desiccated liver powder.
‡ Avg of 2 pools of 4 individual sera. § Avg of 2 determinations each based on pool of 4 livers.

* Armour and Co. † Cortone Merck.

however, the steroid was incorporated into the food, as described by Ershoff(1), in a concentration to provide 6 mg/10 g of diet. Body weights were recorded daily. Blood pressure determinations(3) were made without anesthesia, at the outset and at the conclusion of the experimental period of 2 weeks. Prior to sacrifice food was withheld from all animals for 16 or 24 hours. Then the rats were anesthetized with nembutal and blood pipetted from the incised heart for determination of serum sugar and sodium concentration.‡ Sera were collected individually, and pooled from 4 animals, providing 2 separate pools in each group. At autopsy, body weight, and weights of heart, adrenals, thymus, right kidney, testicular fat body, and liver were recorded. The livers were then collected in dry ice in two pools from each group and total reducing substances (liver glycogen) determined. Chemical and bioassays were carried out on mixtures of liver powder with cortisone acetate.§

In addition to the above experiment, additional data were accumulated on body growth and adrenal weight of groups of 6 to 12 normal rats receiving a low sodium diet(2) with 10% liver supplementation and cortisone acetate orally in a concentration of 2 mg/100 g diet, or subcutaneously in a dosage of 2.0, 1.0 and 0.5 mg daily. The results, combined with data from Groups II and IV in Table I, are presented in graphic form in Fig. 1.

Results. The results are set forth in Tables I and II and in Fig. 1. In the first table, 4 observations relative to growth are notable: 1) Comparison of the weight gains of rats in Group II, given dietary supplementation with liver powder, with those of Group I, indicates that the growth-inhibiting effect of the steroid was prevented by the liver as reported by Ershoff; 2) when the steroid was injected subcutaneously, however, it was seen that the addition of liver to the diet failed to counteract growth retardation (*cf.* Groups III and IV); 3) the addition of liver to the diet was

TABLE II. Effect of Dietary Liver Supplementation on Action of Cortisone Acetate upon Body and Organ Weights.*

Group:	I	II	III	IV	V	VI	VII	VIII
No. in group	8	8	8	8	8	7	8	8
Adrenals	intact				excised			
Cortisone acetate, mg/day	±6	±6	3	3	±6	±6	3	3
Route of steroid admin.	per os	per os	subcut.	subcut.	per os	per os	subcut.	subcut.
Liver in diet, %†	0	10	0	10	0	10	0	10
Body wt, g	123	155	118	112	103	109	106	104
Range	97–142	137–181	110–131	100–125	90–120	92–128	85–115	92–118
Adrenals, mg	22(18)	21(14.9)	18(15.3)	17(14.2)				
Range	16–37	13–26	16–21	16–20				
Heart, mg	635(516)‡	693(446)	667(565)	678(605)‡	603(585)‡	609(560)	627(590)	615(592)
Range	562–696	638–743	555–715	593–785	554–660	553–690	512–690	553–650
Right kidney, mg	638(518)	739(477)‡	665(563)	713(637)	592(574)	684(629)§	650(614)	644(619)
Range	545–784	616–820	534–731	603–811	522–650	610–753	513–795	538–731
Thymus, mg	52(42)‡	61(39)	21(18)	23(21)‡	32(31)	36(33)	17(16)	19(18)
Range	34–70	37–96	14–34	14–30	22–42	28–61	10–28	11–28
Testicular fat body, mg	615(500)	971(626)	895(759)	866(774)	514(499)	472(432)	723(681)	634(609)
Range	420–962	768–1204	521–1250	510–1156	379–676	397–620	462–856	522–699
Liver, g	4.43(361)	5.61(362)	5.46(464)	5.96(532)	3.91(380)	3.88(356)	5.49(517)	5.55(534)
	3.56–5.05	4.82–6.21	4.40–6.55	4.74–7.61	2.95–4.57	3.77–5.50	4.39–6.21	2.95–4.57

* Values in parentheses = wt/100 g body wt. † Armour's desiccated liver powder. ‡ 7 animals, § 6 animals.

‡ For these determinations we are indebted to Dr. D. M. Tennent.

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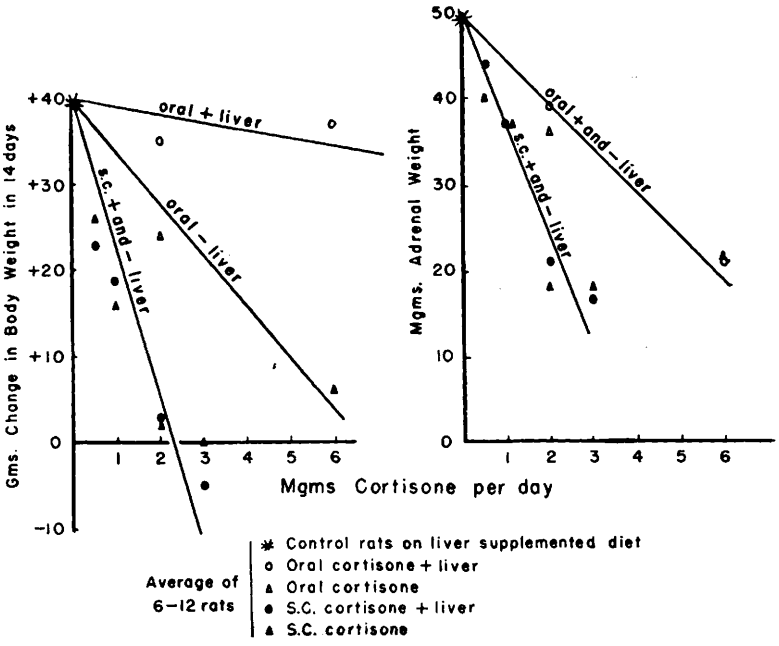

FIG. 1.
Left—Ability of liver to counteract growth retardation due to orally administered cortisone.
Right—Failure of liver to counteract adrenal atrophy due to orally administered cortisone.

without effect upon the growth of adrenalectomized rats given cortisone acetate regardless of the route of administration of hormone (compare Groups V through VIII). 4) Comparison of Groups I and II, neither receiving liver supplement, indicates that the steroid was much more effective in suppressing body growth when given parenterally than orally, since equivalent degrees of suppression were obtained with parenteral doses one-half as large as the oral doses.

Determinations of blood pressure reveal that a definite and comparable degree of hypertension developed in all animals by the end of the 2-week experimental period. It is noteworthy that the elevations in blood pressure in the Group II rats, in which the addition of liver prevented the suppressive effect of cortisone acetate upon growth, were quite as striking as in the other 7 groups.

The terminal serum sodium concentrations were essentially normal except in Groups I, V and VI. In the first group the concentration was abnormally elevated, while in the fifth and sixth groups the levels were significantly reduced. However, levels of serum sodium in adrenalectomized groups receiving liver were comparable to those of rats on the unsupplemented diet. Since the serum sodium level in adrenalectomized rats on low sodium diets bears a relation to the dosage of cortisone(4) the findings suggest the absorption of equivalent amounts of cortisone whether or not the diet was supplemented by liver.

Serum sugar values were definitely elevated in 3 of the 4 groups receiving parenteral cortisone acetate, while this obtained in only one of the 4 groups receiving the steroid orally. Similarly the glycogen content of the livers was markedly greater in the rats receiving parenteral hormone. Again liver failed to counteract the expected rise in blood sugar and liver glycogen due to cortisone.

Organ weights are presented in Table II. In the first 4 groups (animals with intact adrenals), marked adrenal atrophy occurred following administration of cortisone by either route irrespective of whether the diet was supplemented with liver. Other organ weights, especially when expressed in relation to body weight, give evidence of a slightly greater hormonal effect in groups receiving parenteral steroid even though the administered dose was approximately one-

half that given the other animals, *i.e.*, the hearts and kidneys in these groups were heavier as were the testicular fat and liver weights, and the thymus was more strikingly atrophied. None of the anatomical changes typical of cortisone action were reversed by liver feeding.

Data presented in Fig. 1 illustrate that in animals receiving the liver supplement cortisone acetate had no effect upon body growth, whereas when the parenteral route was used, increasing doses of cortisone were accompanied by progressively more severe growth retardation even though the diet contained the same liver supplement. In contrast to the marked differences in the effect of liver supplementation upon growth response to orally and parenterally administered hormone, analogous and progressively more severe degrees of adrenal atrophy obtained in all animals as the dosage increased, regardless of the route of administration of the hormone.

Discussion. In view of the finding that liver feeding failed to alter any of the actions of injected cortisone but did abolish its growth depressing action when the steroid was given orally, it was possible that cortisone may be directly decomposed by some factor in liver. To test this, known quantities of cortisone were mixed with liver powder, re-extracted and assayed chemically (Porter, Silber reaction) and biologically (glycogen deposition in mice). By these methods, undiminished quantities of cortisone could be recovered from the mixtures. Hence a direct action of liver powder upon the steroid appears excluded. It remains possible that some factor is added by the rat's intestinal tract which mediates such an action of liver. This appears unlikely, however, since cortisone fed rats, in which the expected growth retardation was counteracted by addition of dried liver to the diet, exhibited other characteristic effects of chronic steroid administration. Equivalent degrees of hypertension, and adrenal and thymic atrophy occurred in these animals as in rats receiving no liver supplement. Hence the action of liver on body weight gain of cortisone-treated rats does not appear to be part of an over-all diminution in hormonal action.

Ershoff reported that in addition to counteracting growth suppression of cortisone acetate, the addition of liver powder to the diet also counteracts retardation of growth and decreases the mortality which accompanies a variety of unfavorable experimental conditions, *e.g.*, exposure to cold(5), sublethal doses of Roentgen rays(6), exhaustion induced by swimming in cold water(7), effect of drugs such as atabrine(8) or dinitrophenol (9), as well as of hormones such as thyroid (10) and cortisone.

Although there are multiple growth promoting factors in liver powder, among which may be nonspecific appetite stimulating substance, it is perhaps pertinent to contrast the "liver effect" in animals receiving cortisone with that of a single growth promoting substance, somatotropin. The antagonism of growth hormone is evident not only upon body growth but, with the exception of adrenal atrophy, also upon every one of the anatomical changes induced by parenteral administration of cortisone(11,12). This is in contrast to the action of desiccated liver which diminishes none of the cortisone effects except that on growth. Ershoff(13) reports an additional dissimilarity in that growth hormone, unlike liver powder, exerts no effect on growth in animals exposed to cold.

The reason for the absence of the "liver effect" in adrenalectomized rats remains unknown. Since the presence of cortisone did not permit this effect in adrenalectomized animals, it is possible that the activity of other steroids from the adrenals, presumably those of the sodium retaining group, is essential for the "liver effect."

Finally, these studies reveal a marked disparity in the effectiveness of oral and parenteral cortisone acetate in the rat. This has been previously observed by others(14). Even though the oral dose was approximately double that given parenterally, less marked changes occurred with oral administration.

Conclusion. 1. Desiccated liver powder as a dietary supplement to a low sodium diet is effective in counteracting the growth inhibition induced by cortisone acetate in rats only if the steroid is presented orally. It is without demonstrable effect in animals receiving the hormone by injection. It is also without effect in adrenalectomized animals, irrespective

of the route of hormone administration. 2. The prevention of growth inhibition by liver powder is not accompanied by other evidences of diminished cortisone activity, *i.e.*, despite normal growth such animals exhibit physiological, anatomical and biochemical changes of the same degree as do cortisone treated rats without liver supplement.

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In vitro Effect of Autologous Lipemic Plasma on Platelet Suspensions.* (20973)

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It has recently been observed that hyperlipemia, produced by orally or intravenously administered fat emulsion, results in a marked decrease in the number of circulating blood platelets(1). As yet, there is no satisfactory explanation for this phenomenon. It has been postulated that this may be due to intravascular agglutination of platelets. This possibility suggested itself when it was noted that the intravenous injection of heparin during the hyperlipemia resulted in a prompt return of the platelet count toward normal values. This finding offers substantial evidence that platelets are not destroyed during intervals of hyperlipemia but rather supports the concept that they are "clumped" and that heparin promotes redispersion.

Swank's(2) observation that hyperlipemia produced agglutination of red blood cells led us to investigate the possibility that a similar process might prevail with platelets. The following experiments were carried out to determine whether or not platelet suspensions would demonstrate agglutination when incubated *in vitro* with autologous lipemic plasma.

Methods. All glassware used in these experiments was siliconated. A platelet suspension was prepared from 150 cc of whole blood obtained from normal healthy adult males according to the method of Stefanini(3) with minor modification. The donors were then given 250 cc of intravenous fat emulsion† over a 2-hour period. Platelet counts (Rees-Ecker) were obtained on the donor's blood prior to the administration of the Lipomul and at varying intervals during its administration. When a significant fall (40-50%) in the individual's platelet count was noted, a 10 cc blood sample was obtained. The blood was centrifuged and the supernatant plasma removed.

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† Lipomul—supplied by Dr. Robert Heinle of the Upjohn Co.