Alternate Day Corticosteroid Administration and Homograft Rejection in Rabbits* (34037)

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(Introduced by M. M. Reidenberg)

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Harter and associates proposed in 1963 that alternate day corticosteroid therapy was effective in immune diseases and carried a decreased incidence of side effects as compared to sustained corticosteroid administration (1). This type of therapy, as well as other intermittent corticosteroid treatment plans has been well studied with respect to childhood nephrosis and results have been generally encouraging (2–5). In the case of other supposed autoimmune diseases, this method of treatment has not been wholly successful (6–8).

The effect of alternate day steroid administration on immune processes *per se* has been evaluated to only a limited extent. In the present study, we compared the effect on homograft rejection in rabbits of alternate day steroid administration with that of sustained release steroid administration.

Method. The 6-alpha-methylprednisolone injected intramuscularly as the acetate (Depo-Medrol), is absorbed over a period of as long as a week (9). Conjugated with sodium succinate, 6-alpha-methylprednisolone is soluble in water (Solu-Medrol), and is absorbed over a period of about 6 hr (10). These two steroid preparations are otherwise entirely alike in potency and metabolic fate, and are widely used in treating the so-called autoimmune disorders.

Using the method of Medawar *et al.* (11–12) homograft survival was evaluated in 24 New Zealand white rabbits, each weighing 2.0–2.8 kg. These animals were given the above preparations in the following manner. Eight animals received methylprednisolone acetate, 0.5 mg/kg, on alternate days, eight animals received methylprednisolone sodium succinate (0.5mg/kg) intramuscularly on alternate days and eight control animals received an equal volume of saline intramuscularly on alternate days.

Subsequently, eight other animals were given varying amounts of methylprednisolone acetate and methylprednisolone sodium succinate. Two animals received methylprednisolone acetate (1 mg/kg) every other day, two animals received methylprednisolone acetate (2 mg/kg) every other day, two animals received methylprednisolone sodium succinate (1 mg/kg) every other day and two animals received methylprednisolone sodium succinate (2 mg/kg) every other day.

Administration of these preparations was started 4 days before skin grafts were applied. Methylprednisolone sodium succinate was kept frozen in small aliquots and defrosted at the time of use. Methylprednisolone acetate was refrigerated. Homografts wre taken from adult chinchilla rabbits. Sixteen pinch grafts, each about 1 cm^2 were applied to an open graft site on the lateral thorax of each New Zealand white rabbit. Homograft survival was assessed at frequent intervals by observation of the graft site and repeated biopsies of the pinch grafts. When, by microscopic and visual criteria, necrosis of essentially all the epithelium in the graft site had occurred, graft rejection was considered to have reached an end point (9, 10) (Fig. 1). Animals were sacrificed at 28 days. Adrenal glands were then removed, weighed fresh, and prepared for histologic staining.

Results. Methylprednisolone acetate caused a significant increase in homograft survival over methylprednisolone sodium succinate and controls.[1] (Table I). The survival

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[1] $p < 0.01$ by Student's t test (13).

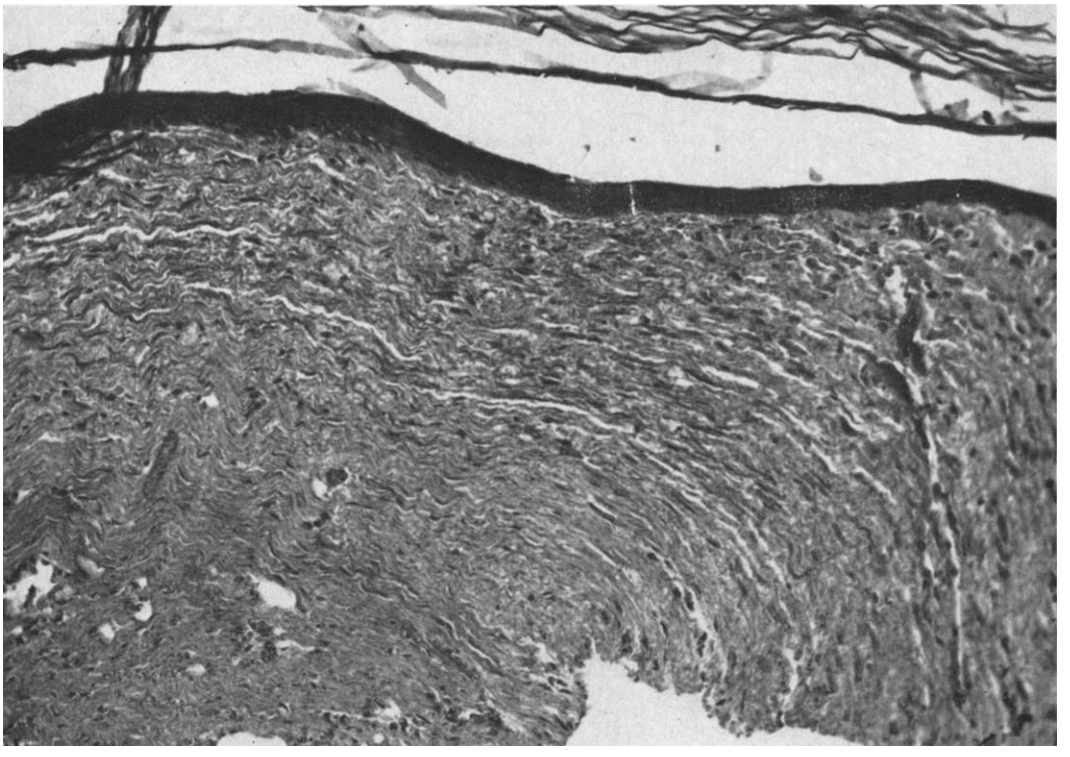

FIG. 1. Histologic changes in homografts in rabbits treated with methylprednisolone acetate or methylprednisolone sodium succinate. (a) Skin biopsy taken at 20 days post-grafting from an animal given, on alternate days, 0.5 mg/kg of methylprednisolone acetate (×200). Epithelium is thin, but normal in appearance. (b) Same animal at 22 days (×370); epithelial degeneration and separation is seen. (c) Same animal at 24 days; only fibrin and granulation tissue remain (×40). (d) Skin biopsy taken at 10 days from an animal given 0.5 mg/kg of methylprednisolone acetate on alternate days (×230). Evidence of early epithelial degeneration. (e) Same animal at 12 days; epithelium necrotic and separated from underlying tissue. Nothing but granulation tissue remained at 14 days.

of skin grafts in animals treated with methylprednisolone sodium succinate was slightly greater than that of controls. Table II shows measurements of parameters associated with other effects of these steroid preparations on the experimental animals. Animals given methylprednisolone acetate lost more weight than animals given methylpredniso-

TABLE I. Effect of Sustained versus Intermittent Steroid Program on Homograft Survival.[a]

Steroid preparation	No. of animals	Alternate day dose (mg/kg)	Mean day of rejection ± SEM
Methylprednisolone acetate	8	0.5	24.8 ± 1.5
Methylprednisolone sodium succinate	8	0.5	11.3 ± 0.8
Saline	8	Equiv vol	10.1 ± 0.8

[a] Preparations were administered in a single dose every 48 hr, so that the administration of methylprednisolone acetate resulted in a sustained high level of steroids and the administration of methylprednisolone sodium succinate resulted in a short period of high blood levels. The difference between methylprednisolone acetate induced survival and other survival times was significant, $p < 0.01$ (13).

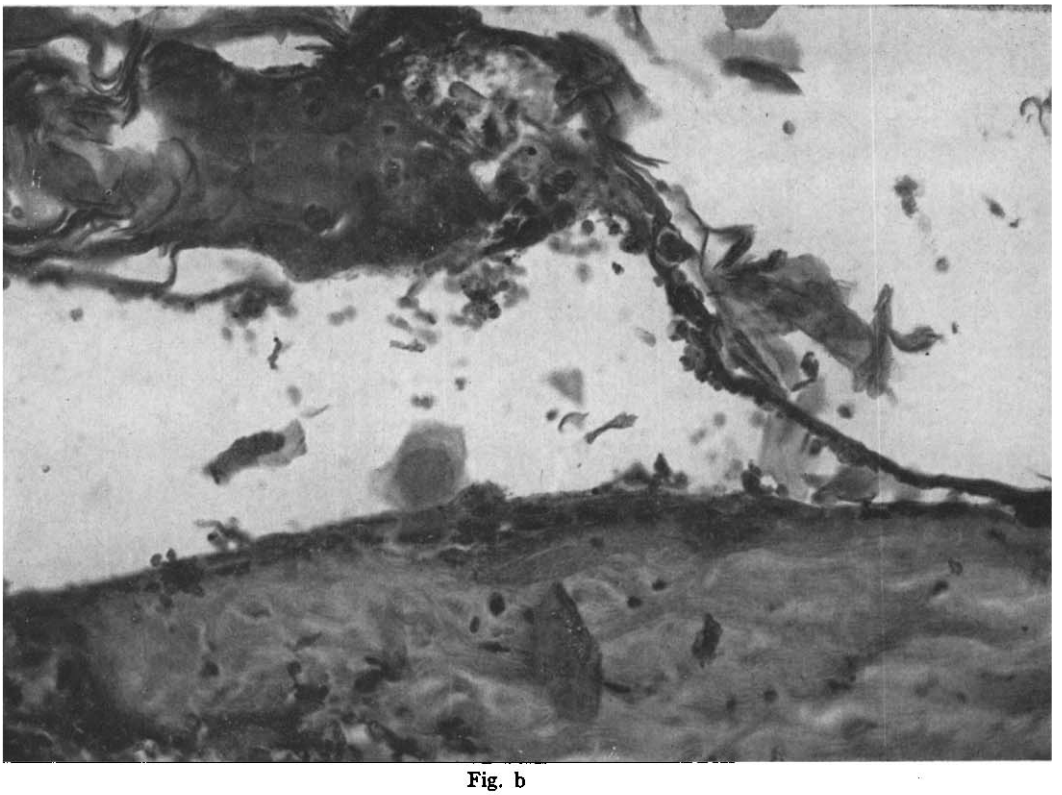

Fig. b

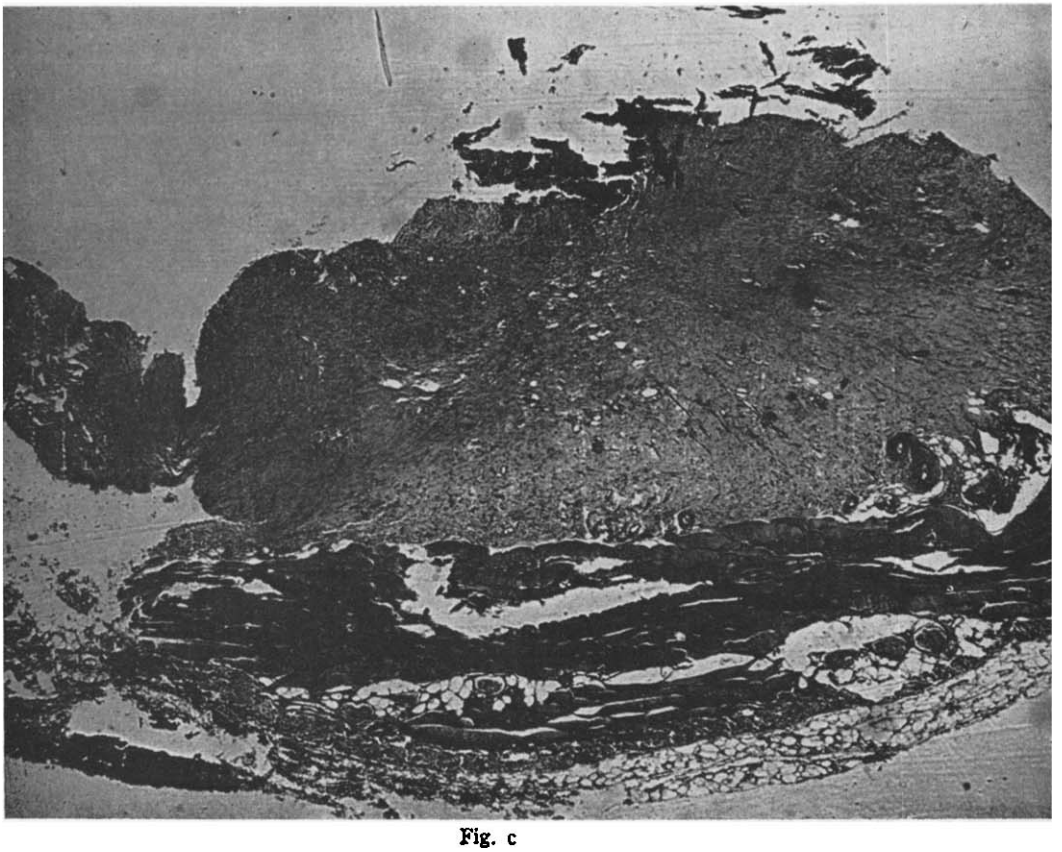

Fig. c

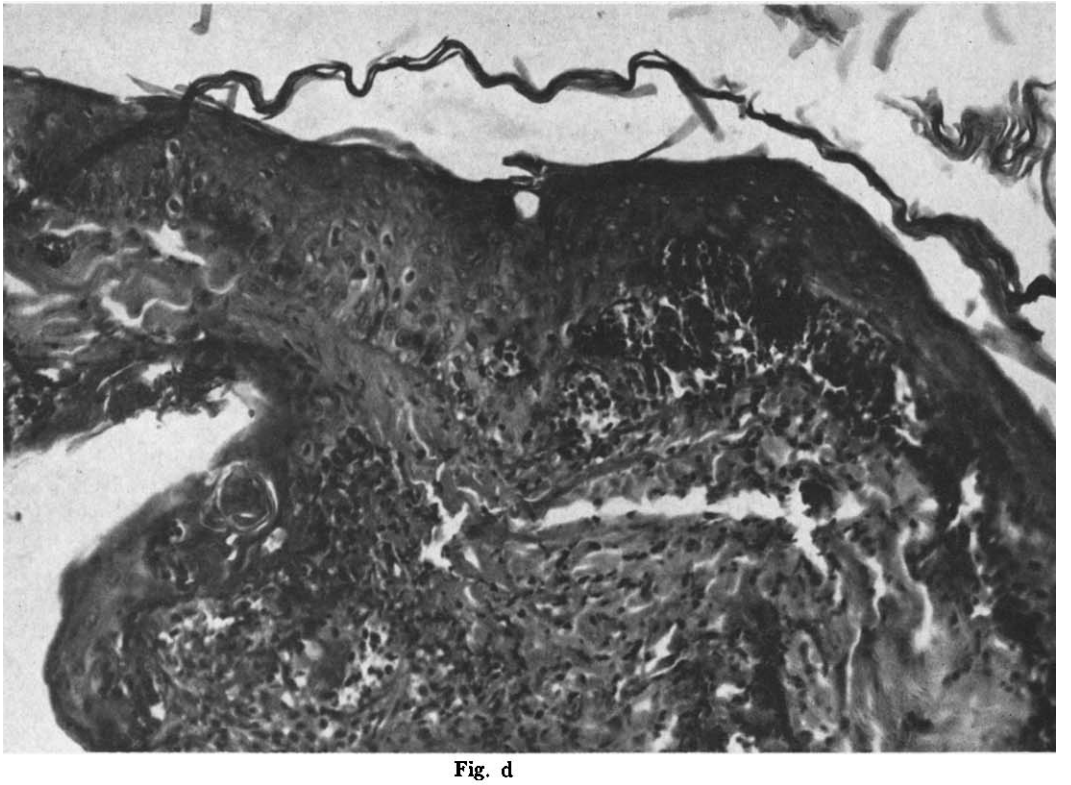

Fig. d

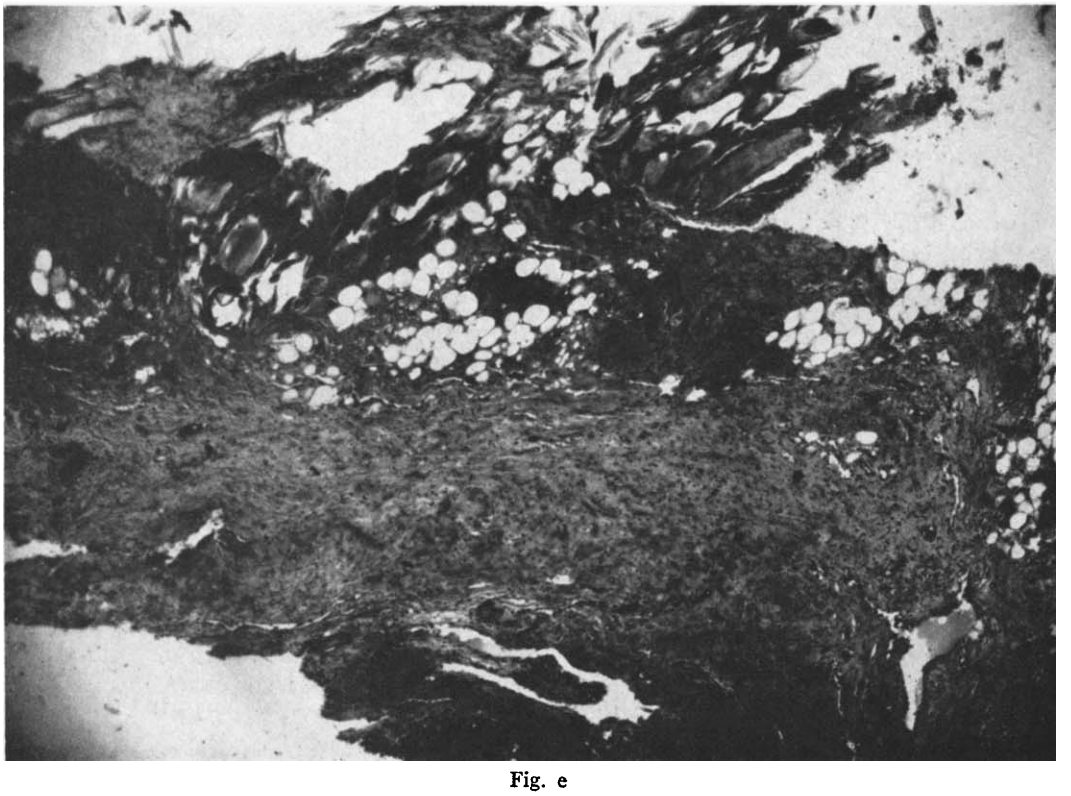

Fig. e

TABLE II. Effect of Sustained versus Intermittent Steroid Program on Body Weight, Adrenal Size, and Adrenal Histologic Characteristics.[a]

Steroid preparation	No. of animals	Av final wt ± SE[b]	Av adrenal wt ± SE[c]
Methlyprednisolone acetate	8	82.5 ± 1.1	27.9 ± 1.2
Methylprednisolone sodium succinate	8	98.1 ± 1.0	37.2 ± 1.7
Saline	8	101.3 ± 1.0	44.1 ± 2.9

[a] Rabbits weighing 2.0–2.8 kg received 0.5 mg/kg of the respective steroid preparation intramuscularly on alternate days. Animals were weighed and sacrificed at 28 days. Weight loss was significantly less for methylprednisolone acetate-treated animals than for other animals, $p<0.01$. Weight loss for methylprednisolone sodium succinate-treated animals was significantly greater than for control animals, $p<0.05$. Adrenal weight was significantly less for methylprednisolone acetate-treated animals than for methylprednisolone sodium succinate-treated animals or controls, $p<0.01$.

[b] Expressed as percentage of initial body weight (statistical comparison was made after logarithmic transformation) (14).

[c] Expressed as milligrams of adrenal weight per kilogram of animal weight.

lone sodium succinate, or animals given only saline solution. Again, animals given methylprednisolone acetate had smaller adrenal glands than animals given methylprednisolone sodium succinate or control solution. Furthermore, on histologic examination the relative size and lipid content of the zona fasciculata and zona reticularis were decreased.

The possibility of some definite effect on homograft survival with a minimum of toxicity led us to attempt to use of larger amounts of methylprednisolone sodium succinate and methylprednisolone acetate. However, methylprednisolone acetate in doses of 1 mg/kg on alternate days and 2 mg/kg on alternate days was uniformly fatal in 3–14 days. Animals given 2 mg/kg of methylprednisolone sodium succinate and to a lesser extent those receiving 1 mg/kg developed side effects. One of the animals given 2 mg/kg of methylprednisolone sodium succinate died at 8 days of gastrointestinal bleeding. The other animal maintained a viable homograft for 13 days, however, appeared sick at the end of the treatment period and showed both a marked loss of body weight and decreased adrenal gland size. Animals given 1 mg/kg of methylprednisolone sodium succinate on alternate days maintained homografts an average of 14 days, but also developed the above side effects, albeit, to a lesser extent.

Discussion. Administration to rabbits of a steroid preparation causing sustained high blood levels led to significantly greater inhibition of an immune process (homograft rejection) than did administration of another form of the same steroid compound in a pattern comparable to that of alternate day steroid administration in humans. Toxicity and adrenal atrophy were less with the alternate day steroid type of program, as one would expect from the results of previous studies involving alternate day steroid therapy in humans (1, 3). It should be noted that higher doses when administered in the intermittent program maintained homograft survival a bit longer, but not to a degree comparable to that obtained in the sustained level steroid program. Corticosteroid administration was quite toxic to these latter animals. Certainly, protein catabolism must have been quite marked. Therefore, it is reasonable to speculate that this slight increase in survival could be due to damaged physiologic processes resulting in decreased breakdown of the steroid compound and effects beginning to approach that of sustained corticosteroid administration.

In 1963, Harter *et al.* reported the use of alternate day corticosteroid therapy in 58 patients with asthma and other steroid responsive disorders (1). These authors felt that giving the steroid drug in one dose every 48 hr was as therapeutically effective as giving

the same amount of medication in divided portions throughout the 48-hr period. In addition, they presented evidence that this schedule of administration caused less toxicity and less adrenal suppression than previously used therapeutic plans. Subsequently, many studies have substantiated the concept that alternate day corticosteroid therapy does indeed result in less adrenal suppression than daily administration of the drug (2, 3, 5, 6). Correspondingly, these reports have also commented on the effects of intermittent steroid administration on various steroid responsive diseases. However, results have been conflicting (6–8). Especially with respect to the collagen diseases, variability in disease activity, paucity of reliable criteria for disease activity or the lack of a sufficient number of patients to study have made conclusions generally somewhat tentative.

Several years before the use of intermittent corticosteroid therapy in humans, Dougherty *et al.* (15) and Ward and Johnson (16) noted that the observable manifestations of these drugs in suppressing inflammation and immune responses seemed to persist after the steroid compound was no longer present. Although much work remains to be done on the basic mechanisms involved and on induction and recovery characteristics before the implications of these observations are fully clarified (17, 18) they provide a theoretical basis for intermittent corticosteroid therapy. Thus, although there was decreased homograft survival in rabbits on an alternate day corticosteroid program, this does not necessarily establish that alternate day corticosteroid therapy is of no value in human disease. Slight differences in the mechanisms involved could allow for differences in response in these studies as compared with those done in some human diseases. As a case in point, the nephrotic syndrome in childhood has been well studied and there seems to be clear evidence that survival in children with this disease who are treated with steroids on an alternate day basis is much better than if they were not treated (2, 4). Most significantly, it would seem that the finding that corticosteroids given on alternate days do not sustain homograft survival to a degree which is comparable to that of sustained corticosteroids does raise the question that alternate day corticosteroid therapy may not be suitable for suppressing manifestations of all types of immune rejection phenomena. Consequently, one should still regard the clinical response of the patient as the criterion of adequate therapy and consider sustained corticosteroid administration in the face of failure to respond to alternate day therapy or in treating the more severe manifestations of collagen diseases.

Summary. The effect of alternate day steroid administration on immune processes *per se* has been evaluated to only a limited extent. In the present study, we compared the effect on homograft rejection in rabbits of alternate day steroid administration with that of sustained release steroid administration. It was found that administration of a steroid preparation causing sustained high blood levels led to significantly greater inhibition of an immune process (homograft rejection) than did administration of another form of the same steroid preparation causing high blood levels only on alternate days. This finding would seem to bear consideration in the decision as to whether to treat autoimmune diseases with sustained high levels of steroids or on an alternate day basis. In addition, observations previously noted with respect to the relation of alternate day versus continuous steroid administration to toxicity and adrenal characteristics were supported by our findings.

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Acetyl CoA Carboxylase and Fatty Acid Synthetase Activities in Liver and Adipose Tissue of Meal-fed Rats* (34038)

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The cytoplasmic malonyl-CoA pathway, catalyzed by acetyl CoA carboxylase and fatty acid synthetase multienzyme complex, is recognized as the major pathway of fatty acid biosynthesis (1, 2). The activity of fatty acid synthetase has been reported to be many times in excess of acetyl CoA carboxylase activity thereby suggesting that the carboxylation of acetyl CoA to malonyl CoA is the rate-limiting step in fatty acid biosynthesis (3, 4). More recently, however, Chang *et al.* (5) have reported the activity of both enzymes to be similar in liver of mice, chickens, and rats.

Meal-feeding, the limitation of access to food to a single, daily 2-hr meal, markedly stimulates lipogenesis in adipose tissue of the rat (6–8). This increased lipogenic capacity is accompanied by an enhanced activity of several enzymes related to fatty acid synthesis, including acetyl CoA carboxylase (9). However, the influence of meal-feeding on the activity of fatty acid synthetase has not been investigated. Consequently, the present experiment was conducted in which the activities of acetyl CoA carboxylase and fatty acid synthetase were assayed in liver and adipose tissue of meal-fed and nibbling (*ad libitum*-fed) rats.

The results presented show that the specific activities of these two enzymes are similar in adipose tissue and liver of the *ad libitum*-fed rat. Also, the activity of both enzymes is significantly higher in adipose tissue, but not liver, of meal-fed as compared to nibbling animals.

Experimental. Male rats of the Sprague-Dawley strain weighing 294 ± 10/(AU ± SEM) were used. The "meal-fed" animals had access to food from 8 AM to 10 AM only whereas the "nibblers" were fed *ad libitum*. Water was available at all times. The composition of the diet has been previously described (10). The rats were maintained on their respective feeding schedules for at least 3 weeks, a period adequate to induce the

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