based on incorporation of radioactive amino acids into C'3 in tissue culture, has suggested that the cell of synthesis is the macrophage (*15*). Macrophages isolated from peritoneal and lung exudates were most active in the synthesis of C'3.

If the reticuloendothelial cells of the liver, the Kupffer cells, were responsible for hepatic production of C'3, since such tissue clearly represents less than half the total reticuloendothelial system of macrophages of the body, one would expect a mixture of recipient and donor C'3 types following transplantation of the liver.

Note added in proof: After submission of this manuscript for publication, a paper by Azen and Smithies (*16*) appeared describing their independent finding of C'3 polymorphism by high-voltage starch gel electrophoresis. On exchanging serum samples, it was established that their $C3^1$, $C3^2$, and $C3^3$ correspond to *C'3 F*, *C'3 S*, and $C'3\ S_{0.6}$, respectively, in our nomenclature (*5*).

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Brown Adipose Cells: Spontaneous Mobilization of Endogenously Synthesized Lipid

Abstract. *Isolated brown adipose cells, devoid of a basement membrane, readily synthesized a variety of lipids from radioactive acetate, a reaction augmented by glucose and insulin. A large proportion of the newly formed fatty acids passed into the incubation medium. In intact brown adipose slices and isolated white adipose cells, most of the synthesized lipid was retained as glyceride esters. The data suggest that the rapid turnover of endogenously synthesized lipid in brown adipose cells is almost totally obscured in studies with intact tissue slices because of interstitial barriers to the egress of fatty acid.*

Conflicting views exist on the lipogenic activity of brown adipose tissue. It has been suggested that the low lipid content in brown fat may be the result of inadequate fatty acid synthesis (*1*), whereas other data have shown that lipogenesis in brown fat in vivo is active, and at times is equal to or greater than that in white fat (*2, 3*). My experiments show that lipogenesis in isolated brown adipose cells was brisk and sensitive to added glucose and insulin, and that a sizable proportion of newly synthesized fatty acid is spontaneously and preferentially released into the incubation medium. In white adipose cells retention is favored.

Male Wistar rats (180 to 200 g) raised on Purina chow were killed by a blow to the head, and the dorsal interscapular fat pad was excised. The pads were examined through a dissecting microscope, stripped of adherent white fat and interdigitating muscle, and cut into 5- to 10-mg pieces. The tissue fragments were added to a 30-ml plastic bottle containing 10 ml of Krebs-Ringer bicarbonate buffer with one-half the recommended concentration of Ca ion and 5 percent dialyzed bovine albumin. The buffer-albumin mixture had been frozen and was gassed to *p*H 7.4 with a mixture of O_2 and CO_2 (19:1) before use. Glucose was present at a concentration of 3 mmole/liter, and 10 mg of collagenase (*4*) was added.

The flask was capped and secured in a water bath (37°C), and the mixture was stirred with a small plastic-coated magnetic bar. Incubation continued until the next pad was ready to be sectioned; for each experiment three to six pads were added this way. If the period of tissue handling exceeded 10 minutes, the pad was discarded. Treatment with collagenase continued for 45 minutes after the last addition of tissue. The incubation mixture was then filtered through surgical gauze moistened with fresh buffer-albumin solution to trap undigested tissue fragments and stromal and vascular elements. The filtrate was centrifuged at 300*g* for 1 minute, and the floating cells were transferred to another vial containing a known volume of buffer-albumin solution. Substrates were added, and the mixture was distributed in 1-ml volumes to plastic vials for final incubation. Methods for lipid extraction and measurement, radioassay, and thin layer chromatography have been described (*5, 6*).

Electron microscopy of the preparation of isolated cells demonstrated structural integrity and preservation of morphological features. Like isolated white adipose cells (*6*), brown cells were not enveloped by a basement membrane.

The conversion of acetate to lipid in isolated brown adipose cells was brisk, and, in the presence of 16 m*M* glucose, it was linear for at least 120 minutes at a velocity of 411 ± 81 nmole per millimole of cell fatty acid per hour (mean $\pm$ S.E.M., $n = 5$). The composition of lipids synthesized by isolated cells differed significantly from that produced by slices of brown adipose tissue (Table 1). In isolated cells, nearly half the newly formed lipid was in free fatty acid, whereas in intact tissue less than 2 percent was in free fatty acids, and over 90 percent of the lipid

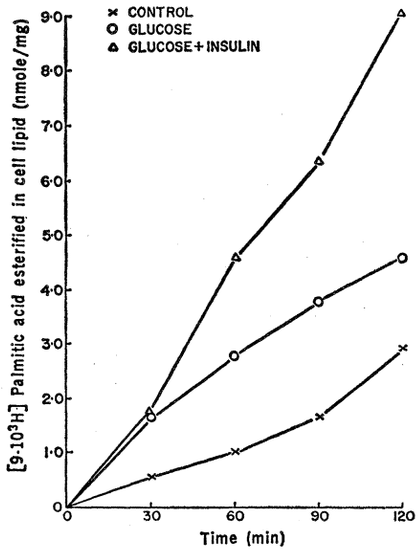

Fig. 1. Fatty acid esterification in isolated brown adipocytes. Brown adipose cells (1.8 mg of cell lipid per flask) were incubated with potassium palmitate labeled with H^3 in the 9 and 10 positions at a final concentration of 71 μmole/liter under conditions similar to that described in the legend to Table 1. Chloroform extracts were prepared (*14*), evaporated, taken up with heptane, and then washed repeatedly with alkaline ethanol to remove unreacted isotopic fatty acid. The washed extracts were assayed for radioactivity and lipid content, and the results are expressed in terms of total cell lipid. Esterification occurred throughout incubation and was augmented by the addition of glucose and insulin.

radioactivity was neutral glyceride. Analysis of saponified products in both preparations showed that over 93 percent of the radioactivity in lipid esters was in the fatty acid moiety and that less than 1 percent of the total lipid radioactivity was in free cholesterol or cholesterol esters.

Since free fatty acids do not ordinarily accumulate in significant amounts within cells, it occurred to me that the high proportion of lipid radioactivity in free fatty acids in the preparations of isolated cells might represent the existence of an extracellular pool of newly synthesized fatty acids. Analysis of the medium and cells separately confirmed this idea (Table 2); 41 percent of the lipid radioactivity was in the bathing medium after 60 minutes of incubation. Glucose and insulin, separately and combined, augmented lipogenesis but did not prevent the accumulation of lipid radioactivity in the incubation medium. Furthermore, over 95 percent of the medium activity was in free fatty acids, whereas over 90 percent of the lipid radioactivity in cells was in glyceride esters. Similar experiments were carried out on slices of brown fat, isolated white adipose cells, and slices of white fat. In the presence of glucose or glucose plus insulin, less than 1.5 percent of the lipid radioactivity was in the incubation medium, and over 95 percent of the total lipid radioactivity was in esterified form.

The partition of lipid radioactivity between incubation medium and cells, as in the experiment shown in Table 2, must be taken as a minimum estimate of total release of newly synthesized fatty acid since reesterification of medium fatty acid occurs throughout incubation. This is inferred from the data in Fig. 1, where palmitic acid added to the medium was esterified by isolated brown cells throughout incubation.

The data show that fatty acid synthesis and esterification occur to a significant extent in isolated brown adipose cells and that these reactions are sensitive to glucose and insulin in a manner similar to that for white adipose cells (*7*). In contrast, Fain *et al.* (*8*) did not obtain significant lipogenesis from glucose in isolated brown adipose cells. The difference in results is probably related to dietary factors because their animals were maintained on a high-fat formula, a nutritional state known to suppress lipogenesis in white fat (*9*) and liver (*10*) and therefore likely to have a similar effect on brown adipose tissue.

Table 2. Distribution of newly synthesized lipid between isolated brown adipose cells and medium during incubation with acetate-1-C^{14}. Isolated brown adipose cells were incubated with acetate-1-C^{14} in plastic vials as described in Table 1, and each flask contained about 4.7 mg of cell triglyceride. The flasks were grouped in triplicate and additions were made in the following final concentrations: glucose, 16 mmole/liter; and insulin, 100 μunit/ml. After a 60-minute incubation, the contents of each group of flasks were combined and filtered through Whatman No. 1 paper. The filter papers were rinsed with 3 ml of fresh buffer-albumin solution, and the lipid radioactivity retained by the filter paper was taken to represent cell lipids; that in the pooled filtrate represented medium lipids.

Additions	Radioactivity (count/min × 10^3)		
	Cells	Medium	Total
O	35.2	25.0	60.2
Glucose	60.8	29.8	90.6
Insulin	53.1	29.5	82.6
Glucose + insulin	157.0	37.5	194.5

The data also lead one to discount the notion (*1*) that the low lipid content of brown fat, compared to white, can be attributed to deficiencies in lipogenic or assimilative processes. The correct explanation would have to take into account the high rate of release of endogenously synthesized fatty acids by isolated brown cells. Although it is not known whether release of fatty acid occurs immediately after its synthesis or whether lipolysis of newly formed glyceride esters precedes release, the phenomenon nevertheless implies a high turnover rate of endogenously synthesized lipid. This idea is in accord with the reported but undiscussed observations of Patkin and Masoro (*2*) that the turnover of lipid newly synthesized from ingested labeled glucose was greater in brown fat than in most other tissues of the rat. Whether variations in turnover of newly synthesized lipid in brown cells is the principal mechanism modulating net lipid accumulation in brown fat cannot yet be decided, since the quantitative significance of circulating lipoprotein lipid as precursor to brown adipose lipid is not known.

The finding that slices of brown fat failed to release newly synthesized fatty acid into the incubation medium, as did

Table 1. The class composition of lipid synthesized by isolated brown adipose cells and slices of brown fat. Isolated cells (27 μmole of cell fatty acid per flask) and tissue slices (75 μmole of tissue fatty acid per flask) were incubated at 37°C for 60 minutes in Krebs-Ringer bicarbonate buffer with 5 percent albumin, containing 0.56 m*M* acetate-1-C^{14} (specific activity 1.33 μc/μm) and 16 m*M* glucose. The gas phase was 95 percent O_2 and 5 percent CO_2. The entire preparation was extracted with a mixture of chloroform and methanol (2:1) and washed once with water and three times with a mixture of chloroform, methanol, and water (3:48:47) (*14*) to insure complete removal of labeled substrate. The class distribution of lipid radioactivity was made by direct assay of silica gel scrapings after thin-layer chromatography of lipid extracts (*6*). Free cholesterol and cholesterol ester contained less than 1 percent of the labeled lipid and are not shown. Abbreviations are: TG, triglyceride; FFA, free fatty acids; DG, diglyceride; PL, phospholipid. Lipid synthesis is measured as labeled acetate converted to lipid (counts per minute per micromole of cell fatty acid per hour).

Preparations	Lipid synthesis	Class composition (%)			
		TG	FFA	DG	PL
Isolated cells	805*	46.6*	45.0	5.6	1.8
Tissue slices	700	80.0	1.6	12.9	4.9

* Average of duplicate incubations.

the isolated cell, is consistent with the observation (*11*) that brown adipose tissue incubated in vitro accumulates free fatty acids within the tissue fragment rather than in the incubation medium because of dense interstitial barriers to the diffusion and egress of fatty acids. Since accumulation of free fatty acid in intact tissue reduces glucose utilization (*12*) and since free fatty acids are causally implicated in the uncoupled oxidative metabolism frequently observed in slices and homogenates of brown adipose tissue (*13*), it is possible that of all the available preparations in vitro, the isolated cell system may best reflect the physiological properties of the parent tissue.

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Antigen-Associated Immunosuppressant: Effect of Serum on Immune Response

Abstract. *Serum from various animal species, including the test animals themselves, inhibits the antibody response of the rabbit to two bacterial antigens, provided that antigen and immunosuppressant interact prior to injection. The degree of immunosuppression is related to the length of incubation in vitro of antigen and serum. Serum does not hinder or destroy the antigenic determinant. Bacterial antibodies do not account for inhibition of the antibody response. Antigen-associated serum components, as yet unidentified, may affect the early events of the immune response.*

The immune response can be inhibited by two fundamentally different mechanisms, the one being specific and the other nonspecific. Specific interference can be accomplished by injection of antigen in suitable physical form and in adequate amounts, leading to immunologic unresponsiveness or tolerance, and by antibodies, particularly of the 7*S* variety, that interfere with antibody production. Nonspecific immunosuppression is effected by damage to the immunologic apparatus, which results from such procedures as thymectomy, x-irradiation, and administration of corticoids and antimetabolites. We now report on a novel type of immunosuppression in which an antigen-associated immunosuppressant, namely, normal serum, interferes with antibody production.

Our experiments followed investigations directed toward elucidation of the lack of immunogenicity of the common antigen (CA) of Enterobacteriaceae (with the exception of *Escherichia coli* O14), first described by Kunin *et al.* (*1*). The endotoxin (lipopolysaccharide) present in the bacteria and supernatants of cultures together with CA inhibits the antibody response to the latter (*2*). Presumably, CA and lipopolysaccharide form a complex that can be separated by ethanol, CA being soluble and lipopolysaccharide insoluble (*3*). Isolated CA proved highly immunogenic. Because various bacterial antigens readily form complexes with certain serum proteins and other substances as well (*4*), the effect of normal serum on immunogenicity of bacterial antigens was studied.

Groups of three albino rabbits, weighing between 2 and 3 kg, were immunized by three intravenous injections, 3 to 4 days apart, of CA from *Salmonella typhimurium* in aqueous solution (*3*) mixed with either normal rabbit serum or phosphate buffer for control purposes. One group was given the mixture of antigen and serum after the mixture was incubated in vitro for 30 minutes at 37°C, and the other group received the mixture prepared immediately prior to injection. The antibody response to CA was measured by means of hemagglutination with CA from *E. coli* O14 as indicator antigen, as described (*5*).

Serum almost completely prevented the antibody response, provided that antigen and serum were incubated prior to injection (Fig. 1). The effect of time for interaction of antigen and serum is evident from the results of an experiment in which the mixtures were incubated for 5, 15, or 30 minutes prior to injection, resulting in mean antibody titers of 1 : 480, 1 : 67, and <1 : 10, respectively. Additional experiments revealed that marked immunosuppressive effects take place when the serum was diluted 1 : 10, but not when used in a dilution of 1 : 100. Even autologous serum, obtained from the test animals before immunization, proved to be immunosuppressive. Inhibition of the antibody response was observed also with rabbit plasma, human serum obtained from healthy subjects, human cord serum, guinea pig serum (*6*), and calf serum (*7*, *8*). Fetal calf serum exerted immunosuppressive effects, although to a lesser extent than calf serum.

The possibility was considered that antibody, possibly by means of a feed-

Table 1. Effect of rabbit serum on antibody response to staphylococcal antigen. Rabbits were injected intravenously on days 0, 3, and 7 with 1 ml of mixtures of staphylococcal antigen and buffer or rabbit serum in various dilutions. Mixtures consisted of one part of culture supernatant and nine parts of serum or buffer. Antibody titers were measured by hemagglutination, with antigen from *Bacillus subtilis* as indicator.

Materials for immunization	Mean hemagglutinin titers (reciprocal) at day				
	0	7	10	14	21
Antigen + buffer	<10	907	3627	3520	3467
Antigen + 1:1 serum	<10	13	13	13	10
Antigen + 1:10 serum	<10	120	400	960	320
Antigen + 1:100 serum	<10	120	1920	3200	800