TECHNICAL PAPERS

Differences in Physiological Activity in Brown and White Fat as Revealed by Histochemical Reactions[1]

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Since the discovery by Hoffmann and Wertheimer (*5*) that fat consumes oxygen, subsequent investigations have disclosed that adipose tissue possesses a number of biochemical activities not previously suspected. Recent experiments by Schoenheimer (*7*), using isotopic compounds as markers, have shown that lipids of the fat depots are not static reserves but are constantly subject to a variety of highly complex chemical reactions of synthesis, degradation, and interconversion. These findings have stimulated renewed interest in the biochemistry and histology of this tissue, so long regarded as metabolically inert. In addition to the white or yellow adipose tissue which occurs in all mammals, many species possess gland-like masses of so-called brown fat in the interscapular, axillary, and inguinal regions. The function of brown adipose tissue is obscure, but evidence is accumulating which suggests that it is quite different from that of white fat. It is known to consume more oxygen than white fat (*1*). Indeed, computed as fat-free tissue, its respiration is as great as that of kidney, and it is as active in oxidizing succinate and pyruvate. It contains cytochrome C and cytochrome oxidase and is rich in ascorbic acid and diphosphothiamine, according to Hook and Barron (*6*). We have been interested in comparing the two types of fat with regard to their histochemical reactions for glycogen and lipase.

Glycogen is not found in the adipose tissues of rats in a normal nutritional state. It can, however, be demonstrated in the fat cells of animals being refed with carbohydrate after a period of fasting (*2*). We have found that it is also possible to cause the deposition of glycogen in adipose tissue by the injection of insulin. Whichever method is used, glycogen occurs in brown fat in much greater quantity than in white fat. Animals whose thyroid function has been abolished by thiouracil, and animals which have been castrated, deposit distinctly more glycogen in their brown fat depots than do normal animals under the same experimental conditions. Hence, the amount of glycogen laid down in brown adipose tissue appears to be under the influence of the ductless glands. Similar endocrine effects upon white fat are suspected, but the glycogen content of this type of fat is so small that significant quantitative differences have not been detected by histological methods.

The histochemical reaction of Gomori (*3*) for lipase was also applied to both types of fat, using as substrates Tween 40, Tween 60, and Product 81. Preliminary observations on the adipose tissues of well-nourished rats indicate that the subcutaneous white fat of the back is devoid of stainable lipase, while the subjacent interscapular brown fat contains a considerable amount of the enzyme uniformly distributed in the cytoplasm between the lipoid vacuoles. The previous observation of Gomori (*4*), that white fat has a negative histochemical reaction for lipase, is thus confirmed, while the occurrence of this enzyme in brown adipose tissue has been demonstrated histologically for the first time.

Further studies on the variations of lipase content in starvation and hibernation are being undertaken.

[1] This work was done in part under a grant from the American Cancer Society on the recommendation of the Committe on Growth of the National Research Council.

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High-Efficiency Counting of Long-lived Radioactive Carbon as CO_2[1]

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The recent availability of long-lived radioactive carbon (C^{14}) from the Manhattan District (*7*) in relatively large quantity has tended to emphasize the difficulties inherent in the available methods of measurement of this important isotope. It has been desirable to adapt the Geiger-Müller counter to this measurement. Ionization chambers are not simple instruments to use when connected with sensitive electrometer circuits. Henriques and Margnetti (*4*) have recently described an ionization chamber used with a Lauritzen electroscope which shows high sensitivity. However, the limiting sensitivity of any method of measurement is determined by the statistics of the backgrounds, which are fundamentally different in counters and ionization chambers. The advantage lies with the counter, as it counts only events, while the ionization chamber integrates the total ionization produced by each event of each kind (*2*). Counting C^{14} from some solid compound of carbon with thin-window β-counters is at best highly inefficient (*8*) because of the very low energy of the

[1] The research described in this paper was supported in part by Contract N5 ori-78, U. S. Navy Department. The author acknowledges his obligation to Prof. R. D. Evans, who instigated this investigation and made it possible; to Mr. R. G. Fluharty, who gave much help on the electrical circuits; and to all the members of the Radioactivity Center at M.I.T. for their time, advice, and cooperation.

β-ray emitted (E_{max} = 0.145 Mev). Some advantage may be gained by inserting the solid compound within the counter to avoid absorption of the β-rays by the window, as reported by Ruben and Kamen (*6*), but the efficiency is still limited by the high self-absorption of the β-ray within the solid compound.

In order to avoid the problems associated with the low energy of this disintegration, the obvious solution is to convert the C^{14} to some gaseous compound and use the sample to be measured as the filling gas of the counter. This will allow the handling of large samples with high efficiency. Under these circumstances virtually all of the β-disintegrations will create ionization within the sensitive region of the counter.

TABLE 1

DESCRIPTION OF THE COUNTER TUBES

Counter No.	Length (cm.)	Inside diameter (cm.)	Total volume (ml.)	Cathode length (cm.)	Cathode volume (ml.)	Total volume/ cathode volume
18	33	3.8	316	28	301	1.050
19	16	1.2	18.1	13.0	13.5	1.340
20	16	2.1	60.5	14.1	48.8	1.240
22	21	2.1	74.2	18.4	63.7	1.165
23	31	2.1	113.3	28.6	99.2	1.142

The only gaseous compound of carbon into which almost any other carbon compound could be readily converted is CO_2. However, it has been reported several times that CO_2 is unsuitable as a counter-filling gas because of the complete lack of a plateau in a counter filled with this gas. There are two possible ways to surmount this difficulty: The chemical preparation of the sample may be carried one step further by converting the CO_2 to some other gaseous compound which will have the desirable counting characteristics, or the electrical discharge characteristics of CO_2 may be modified by adding a second component to the counter. As a result of a study of the effect of contaminants on the discharge characteristics of CO_2 (*1*), carried out in conjunction with the development of a gaseous counting method for C^{14}, a set of circumstances has been found whereby C^{14} may be conveniently and reproducibly measured with high efficiency. Helium, alcohol, ammonia, carbon disulfide, methylene chloride, methyl iodide, ethyl bromide, chloroform, acetone, freon, pyridine, and water have been tried as additives to CO_2, carbon disulfide being by far the best.

The G-M tubes are conventionally designed glass envelope gamma counter type with a 6-mil. tungsten center wire and a cathode of silver chemically deposited on the inner wall and then coated with colloidal graphite. They are tubulated near one end with a stopcock and ground joint for filling. Counters of a wide range of sizes are found to work well. Those used are described in Table 1. The filling gas is the sample of active CO_2 at any pressure from 10 to 50 cm. Hg with, in all cases, sufficient carbon disulfide vapor to represent 2 cm. pressure within the counter tube. Threshold voltages range from 1,800 to 4,500, depending on counter diameter and CO_2 pressure. The plateau starts at 80–90 volts above threshold, extends for 200 volts, and has a slope of the order of 2 per cent/100 volts. The optimum operating range is about 160 volts above threshold. These counter tubes have been used in conjunction with a stabilized high-voltage power supply having an output up to 5,000 volts, modified Neher-Pickering preamplifier, scale of 128, Cenco register, and electric timer.

Under these conditions it is found that within these pressure limits the measured counting rate per unit amount of active CO_2 is completely independent of the total pressure of CO_2 and is thus a function only of the amount of activity within the counter tube. It is further found that, if the counting rate is corrected by the ratio of the total volume of the counter to the volume defined by the cylindrical cathode, all counter tubes within the above size range show the same response to equal amounts of activity within the counters. The nonlinearity correction for these counters is relatively large, amounting to 12 per cent loss at 10,000 c.p.m. This correction is made from a calibration curve prepared by adding increment amounts of active CO_2 to a counter tube and plotting observed counting rate less background against arbitrary units of active CO_2 in the counter tube.

TABLE 2

ACTIVITY MEASUREMENTS

Sample No.	C^*O_2 (micromoles)	CO_2 (millimoles)	Counter No.	CO_2 pressure in counter (cm. Hg)	Threshold voltage	Observed counting rate (c.p.m.)	Background rate (c.p.m.)	Linearity correction factor	Final corrected counting rate	c.p.m. per μ mole C^*O_2
1	42.2	5.67	18	33.8	4,060	2,090	320	1.000	1,860	44.0
2	36.2	0.159	19	20.1	2,920	1,420	40	1.000	1,850	51.1
3	56.1	3.10	20	9.8	4,320	2,200	70	1.005	2,655	47.3
4	131	1.18	23	21.6	3,555	5,230	159	1.065	6,170	47.1
5	201	1.15	22	34.0	3,720	7,130	99	1.138	9,320	46.3
6	254	3.20	18	20.5	3,540	9,840	301	1.203	12,040	48.8
7	348	0.159	19	52.3	4,390	9,550	39	1.295	16,500	47.4

The measurements presented in Table 2 were made on samples prepared by mixing measured amounts of inert and active CO_2. The samples of activity were all drawn from the same bulb containing a preparation of radioactive CO_2. The prepared sample was distilled into a counter tube attached to the glass system with a ground joint by immersing one end of the counter tube in liquid nitrogen. The proper amount of CS_2 vapor to represent 2 cm. pressure in the counter was then added by means of a "doser," after which the counter was detached from the line, allowed to warm to room temperature, placed in a lead housing, the preamplifier leads attached, and the counting rate determined at 160 volts above threshold. Background rate is determined in the same counter filled with inert CO_2.

The final corrected counting rate is obtained by subtracting background from the observed rate, multiplying this by the ratio of the total volume of the counter to the cathode volume from Table 1 to correct for that fraction of the sample that is not within the sensitive volume of the counter tube, and then correcting this figure for the nonlinearity of the counters with the factor taken from the empirically determined curve previously referred to. The data in the last column of Table 2 show that, with an average deviation of 3 per cent, the system allows the direct comparison of activities of a large range of sample sizes. The sample size range from 0.1 to 9 millimoles of carbon is represented by 10 cm. pressure in the smallest counter to 50 cm. in the largest. In terms of $BaCO_3$, on

which measurements are usually made by solid counting techniques, this represents 20 mg.–2 grams. Sample 7, when removed from the counter, precipitated as $BaCO_3$ on a 2-cm. filter, and measured on a bell-type β-counter (*3*) with a 4-mg./cm.^{2} window, gave an observed counting rate, less background, of 50 c.p.m. This is 2.5 × background as against 250 × background on the sample when measured as a gas.

While there is no proof in the data that this is an absolute disintegration rate measured by these counters, the fact that the ionizing events registered are those that take place within the cylindrical volume defined by the cathode strongly suggests that the final corrected counting rate is indeed the absolute disintegration rate of the activity within the counter tube. If there were losses, one would expect, from the nature of the discharge avalanche that constitutes the pulse from the counter tube, that these would be greater in the tube of larger diameter. The data on counters Nos. 18 and 19 demonstrate that this is not the case. Thus, measurements on C^{14} made in this way, together with the mass spectrometer analysis of C^{14} activity preparations, will allow a determination of the presently quite uncertain decay constant of this isotope with an accuracy far greater than is possible by solid counting (*5*), although this will probably not approach that possible with modern ionization chamber methods after the mean energy of the β-ray is obtained from the spectrum.

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Effect of Flavonols on the Bacteriostatic Action of Dicoumarol

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Goth (*3*) reported that dicoumarol possessed bacteriostatic action toward certain bacteria which was not antagonized by 2-methyl-1,4-naphthoquinone (vitamin K). This would indicate that dicoumarol exerts its bacteriostatic activity through a mechanism different from that by which it induces hypoprothrombinemia and hemorrhage. In connection with some investigations in progress at this Laboratory (*2*), it was of interest to determine the effect of compounds containing the γ-pyrone structure on the antibacterial action of dicoumarol. For this purpose we have used the flavonol glycosides, rutin (*1*) and quercitrin, and the aglycone, quercetin. The effect of rutin was of especial interest, since it has pronounced physiological activity in diminishing the tendency to hemorrhage by restoring fragile capillaries to normal (*4*, *5*).

The tests were made in nutrient broth (peptone, 0.5 per cent; beef extract, 0.3 per cent; and sodium chloride, 0.5 per cent) adjusted to pH 6.95. Nutrient broth solutions containing desired concentrations of dicoumarol and flavonols were dispensed in 5-ml. quantities in test tubes, sterilized by autoclaving at 15 pounds for 15 minutes, inoculated with 0.01 cc. of a 16-hour broth culture of *Staphylococcus aureus* (F.D.A. 209P), and incubated at 37° C. The antagonistic effect of the flavonols on dicoumarol was determined by using a Klett-Summerson photoelectric colorimeter to measure the density of bacterial growth in the presence of increasing quantities of the flavonols.

TABLE 1

Antagonistic Effect of Rutin, Quercitrin, and Quercetin on the Bacteriostatic Activity of Dicoumarol Toward *Staph. aureus*

(Expressed as turbidity readings on Klett-Summerson colorimeter scale)

		Dicoumarol (mg./ml.)			
		0.0	0.02	0.04	0.08
Rutin (mg./ml.; 22 hrs. at 37°C.)	0.0	62	47	0	0
	0.01	66	50	0	0
	0.05	62	58	26	0
	0.5	62	55	59	42
Quercitrin (mg./ml.; 15 hrs. at 37°C.)	0.0	47	27	0	0
	0.05	48	48	35	0
	0.1	52	53	39	21
	0.5	44	38	30	29
	1.0	17	16	13	14
Quercetin (mg./ml.; 19 hrs. at 37°C.)	0.0	45	29	0	0
	0.01	48	42	16	0
	0.05	38	22	13	0
	0.10	0	0	0	0

The results in Table 1 show that all three flavonols were capable of neutralizing the bacteriostatic action of dicoumarol. The inhibitory effect of 0.04 mg./ml. of dicoumarol was overcome by 0.05 mg./ml. of rutin and completely neutralized by 0.5 mg./ml. Higher concentrations of dicoumarol required increased amounts of rutin to show proportional antagonism. Rutin *per se* does not appear to have any effect on the growth of *Staph. aureus*.

Quercitrin was somewhat less effective than rutin as an antagonist toward dicoumarol. This may be partly due to the fact that in high concentrations quercitrin exhibits toxicity toward *Staph. aureus*. In concentrations up to 0.1 mg./ml. it showed increasing antagonism toward dicoumarol; however, above this value the toxic effect began to show up, and at 1.0 mg./ml. there was a 64 per cent inhibition in the growth of *Staph. aureus*.

Quercetin was the least effective of the three flavonols tested. It did not overcome the bacteriostatic effect of 0.08 mg./ml. of dicoumarol, and showed only partial antagonism to the lower concentrations. It exhibited considerable toxicity toward *Staph. aureus*, completely inhibiting the growth in a concentration of 0.1 mg./ml.

The antibacterial action of quercitrin is probably due to the presence of some quercetin from the hydrolysis of the rhamno-

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