of various tissues and behavioral aspects of reproduction. The ability for full reproductive capacity to be realized with PMS primed immature rats allows for sequential analysis of deficits or defects and their interrelationships.

Summary. Subcutaneous injection of 10, 100 or 1000 μg of testosterone prop. in a single dose into female rats on the fifth day of life prevented luteinization of the ovaries after 10 days of parabiosis with castrated male rats. Injection of 10 or 100 μg of testosterone propionate on days 5, 7 or 9 of life also prevented luteinization of the ovaries, but animals injected with the same amount of hormone on day 13 had luteinized ovaries. Animals injected with 1 mg of testosterone propionate on day 13 formed no corpora lutea when put in parabiosis at 26 days of age. Animals joined at 36 days of age, however, did form corpora lutea. Animals treated with hormones in the early postpartum period and injected with PMS at 30 days of age did not ovulate. Animals treated with 10 μg of testosterone propionate on day 5 and injected with 16 I.U. of PMS on day 30 did not have corpora lutea at 34 days of age or at 58 days of age.

1. Pfeiffer, C. A., *Am. J. Anat.*, 1936, v58, 195.
2. Wilson, J. G., Hamilton, J. B., Young, W. C., *Endocrinology*, 1941, v29, 784.
3. Wilson, J. G., *Anat. Rec.*, 1943, v86, 341.
4. Noumura, T., *J. Fac. Sci., Univ. of Tokyo*, IV, 1958, v8, 317.
5. Barraclough, C. A., *Endocrinology*, 1961, v68, 62.
6. Kallas, H., *Compt. Rend. Soc. de Biol.* 1929, v102, 280.
7. Kupperman, H. S., Meyer, R. K., Finerty, J. C., *Am. J. Physiol.*, 1942, v136, 293.
8. Biddulph, C., Meyer, R. K., PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 92.
9. Witschi, E., Levine, W. T., *ibid.*, 1934, v32, 101.
10. Cole, H. H., *Am. J. Anat.*, 1936, v59, 299.
11. ———, *Am. J. Physiol.*, 1937, v119, 704.
12. Everett, J. W., Sawyer, C. H., Markee, J. E., *Endocrinology*, 1949, v44, 234.
13. Everett, J. W., Sawyer, G. H., *ibid.*, 1949, v45, 581.
14. Strauss, W. F., Meyer, R. K., *Science*, 1962, v137, 860.
15. Bunster, E., Meyer, R. K., *Anat. Rec.*, 1933, v57, 339.
16. Everett, J. W., *Endocrinology*, 1948, v43, 399.
17. ———, *Sex and Internal Secretions*, W. Young, Ed., Williams and Wilkins Co., Baltimore, 1961, v1, 497.
18. Price, D., Ortiz, E., *Endocrinology*, 1944, v34, 215.
19. Greer, M. A., *ibid.*, 1953, v53, 380.
20. Johnson, D. C., Witschi, E., *Cancer Research*, 1961, v21, 783.
21. Pfeiffer, C. A., *Yale J. Biol. Med.*, 1942, v14, 619.
22. Boling, J. L., Blandau, R. J., *Endocrinology*, 1939, v25, 359.
23. Wilson, J. G., Young, W. C., *ibid.*, 1941, v29, 779.
24. Gale, S. C., McCann, S. M., *J. Endocrinol.*, 1961, v22, 107.

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Effects of N-Ethyl Maleimide and Insulin upon Adipose Tissue. (28197)

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If the action of insulin is dependent upon the integrity of disulfide and/or sulfhydryl groups on the cell surface, alterations of these groups should interfere with the response of the cell to insulin. There have been conflicting reports referable to this question, particularly in the case of rat diaphragm(1) and heart muscle(2) as well as adipose tissue(3, 4). The present report concerns the effects of N-ethyl maleimide (NEM), a sulfhydryl blocking agent, the response of the rat epididymal fat pad to insulin.

Methods. Modifications of the rat epididymal fat pad assay for insulin-like activity

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TABLE I. Effect of Varying Concentrations of NEM on Glucose Oxidation in Fat.

Insulin concentration μunits/ml	$C^{14}O_2$/mg fat/2 hr NEM 0	NEM 10^{-4}M	NEM 5×10^{-4}M	NEM 10^{-3}M
0	1.92 (28)*	1.87 (8)	.77 (8)	.40 (8)
31	3.10 (28)	3.48 (8)	1.26 (8)	.28 (8)
250	10.52 (27)	12.06 (7)	3.28 (8)	.49 (8)
500	17.04 (26)	13.64 (8)	6.05 (8)	.64 (8)

* No. of samples incubated.

(5) were used, in which quadruplicate samples of tissue were incubated for each expermental point. Glucose uptake was expressed as counts per minute of $C^{14}O_2$ per mg fat (wet weight), with the radioactivity arising from utilization of I-C^{14} glucose (0.1 μC/incubation flask). Insulin in concentrations as low as 31 μunits/ml produces significant increments in $C^{14}O_2$.

Results. A. The first group of experiments was performed to determine the effect of varying concentrations of NEM on glucose metabolism in fat. Tissue samples (approximately 100 mg each) were incubated at 37°C for 2 hours in the presence of 0, 31, 250, or 500 μU insulin/ml with and without varying concentrations of NEM (10^{-3}-10^{-4}M). When present, NEM was added 20 minutes prior to addition of insulin. The data in Table I clearly show that in the absence of insulin, 10^{-3}M NEM completely inhibits, that 5×10^{-4} NEM partially inhibits, and that 10^{-4} M does not inhibit conversion of I-C^{14} glucose to CO_2. At similar concentrations of NEM it appears that the tissue response to insulin is also blocked. B. A second group of experiments was designed to describe certain features of the kinetics of the inhibition caused by NEM. Six assays were performed. In each assay, a range of concentrations of NEM from 10^{-4} to 10^{-3}M was employed with a single concentration of insulin. NEM was added 20 minutes before insulin in 3 assays and insulin added 20 minutes before NEM in 3. Insulin concentrations were 31 μ units in the first assay, 500 in the second, and 50,000 μunits/ml in the third. The velocity of the reaction was obtained by determination of $C^{14}O_2$/mg fat/2 hours. Fig. 1 illustrates the linear relationship of $C^{14}O_2$/mg to time over a 2-hour period, and justifies the use of this parameter as a rate. The results may be seen in Fig. 2, in which the data are plotted as $\frac{VI}{V} = \frac{\text{(inhibited velocity)}}{\text{(uninhibited velocity)}} = \frac{(C^{14}O_2+\text{NEM})}{(C^{14}O_2 \bar{s}\ \text{NEM})}$ *vs* Log Inhibitor Concentration (NEM). The degree of inhibition of glucose metabolism is dependent upon the concentration of NEM, regardless of concentration of insulin present. This appears to be the case whether NEM is added prior to or subsequent to insulin and suggests that insulin and NEM probably do not compete for the same binding sites. In fact, at higher concentrations of insulin (50,000 μunits/ml) greater inhibition rather than lesser was observed.

Treatment of tissues with insulin prior to NEM results in *exaggeration of the inhibition.* Significant inhibition occurred at 10^{-4}M NEM which was not inhibitory when NEM was added prior to insulin. Further, this concentration of NEM did not inhibit endogenous glucose metabolism (samples without insulin) while inhibiting the response of the fat pad to insulin (31, 500, and 50,000 μ units/ml). C. In the third group of experiments, an attempt was made to reverse the inhibition caused by 5×10^{-4}M NEM. Incubation was begun as previously described, but 30 minutes later, the NEM-containing medium was removed from the flask with a syringe and was replaced by medium containing either 250, 500, or 50,000 μU/ml of insulin with no inhibitor. The 2-hour incubation was then completed.

In one experiment, cysteine was added at 30 minutes to make the final concentration 10^{-3}M. Samples of $C^{14}O_2$ were taken after 15, 30, 60, and 120 minutes of incubation to determine degree of inhibition with time.

Table II shows that all attempts to reverse inhibition were unsuccessful. D. The final group of experiments was designed to determine whether addition of NEM and/or the sequence of addition relative to insulin altered the binding of insulin to fat. Tissues were incubated in 2000 μU/ml of high specific activity I^{131} insulin with and without 5×10^{-4} NEM. NEM was added prior to and subsequent to insulin. Following incubation, tis-

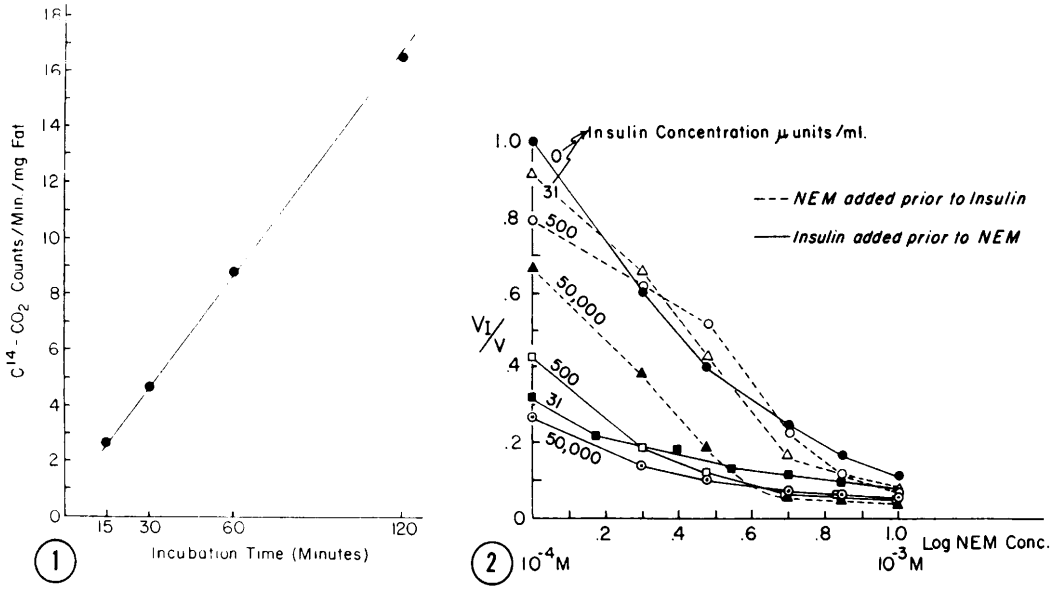

FIG. 1. Conversion of I-C^{14} glucose to CO_2 by rat adipose tissue in presence of 500 μunits/ml insulin.

FIG. 2. Relationship between insulin concentrations, sequence of additions, and inhibition produced by NEM.

sues were blotted, rinsed 4 times in large volumes of distilled H_2O, hydrolyzed in 0.5 ml 30% KOH and I^{131} was counted in a well-type scintillation counter.

Control tissues in the absence of NEM bound 9.0% of the I^{131} insulin counts per g/ fat 2 hours. 9.6% of counts was bound per gram of fat when NEM was added prior to insulin and 8.4% was bound when insulin was added prior to NEM. These data further support the contention that NEM does not bind the same site as insulin. If this were the case, less I^{131} insulin would be bound when NEM was added prior to insulin. In addition, alteration in binding of insulin does not appear to be responsible for the enhanced inhibition occurring when insulin is added prior to NEM.

Discussion. The data presented show that under special conditions a sulfhydryl blocking agent (NEM) can inhibit the stimulatory effect of insulin on glucose metabolism in fat tissue without inhibiting endogenous glucose metabolism. This implies a surface action of NEM in the inhibition rather than an intracellular effect, and suggests a relationship between insulin action and SH-S-S groups on the cell surface. No evidence is available, however, that the binding of insulin to the tissue is impaired by the blocker. Further.

TABLE II. Irreversibility of NEM Inhibition.

Insulin μunits/ml	250	250	250	250	500
Initial					
NEM - Molarity	0	5×10^{-4}	5×10^{-4}	5×10^{-4}	5×10^{-4}
At 30" Insulin	250	250	250	50,000	500
NEM	0	5×10^{-4}	0	0	5×10^{-4}
Cysteine	0	0	0	0	10^{-3}M
Incubation time in min			cpm of $C^{14}O_2$/mg fat		
15	2.66	.48*	.12	.33	.26
30	4.68	.70	.09	.24	.19
60	8.82	.91	.14	1.05	.38
120	16.50	1.05	.30	.76	.31

* In all cases values represent 4 samples incubated.

the mechanism is not a simple competition for SH or S-S groups, since the concentration of insulin added has little effect on the inhibitory concentrations of blocker. The rather interesting finding that addition of insulin prior to blocker results in greater inhibition in the fat pad has also been observed in isolated rat diaphragm muscle(1) though not in perfused rat heart(2). No obvious explanation for this is apparent. One can hypothesize that in the process of binding insulin to tissue, an increased number of binding sites are made available for the NEM or that insulin in some manner increases the permeabilty of the tissue to NEM resulting in high concentrations of NEM intracellularly.

Summary. N-ethyl maleimide, a sulfhydryl blocking agent, is capable of inhibiting the response of adipose tissue to insulin. The inhibitory mechanism is not competitive. Although the effect appears to be upon the cell surface, the binding of insulin to the tissue is not impaired.

1. Carlin, H., Hechter, A., *J. Biol. Chem.*, 1961, v237, 1371.

2. Cadenas, E., Kaji, H., Park, C. R., Rasmussen, H., *ibid.*, 1961, v236, 63.

3. Mirsky, J. A., Perisutti, G., *Biochem. Biophys. Acta*, 1962, v62, 490.

4. Lynn, W. S., Earnhardt, E., Brown, R., *J. Clin. Invest.*, 1961, v40, 1059.

5. Renold, A. E., Martin, D. B., Dagenais, Y. M., Steinke, J., Nickerson, R., Sheps, M. C., *ibid.*, 1960, v39, 1487.

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Relation of Molecular Size of Dextran to its Effects on the Rheological Properties of Blood.* (28198)

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In recent studies with dextran as a plasma expander in the treatment of shock(1) and in extracorporeal circulations(2), it has been reported that low molecular dextran is more effective than the higher molecular fractions in promoting and maintaining flow through the capillaries and small vessels, seemingly by lowering the viscosity of the blood and reducing the tendency of the red cells to aggregate and stagnate in the small vessels(1). The present experiments were undertaken to test the possibility that low molecular dextran actually lowers the viscosity of the blood in some way not explained by changes in the red cell concentration.

Methods and procedures. Four preparations of dextran with mean molecular weights of 15,000; 45,000; 75,000 and 250,000 (provided by Pharmachem Corp., Bethlehem, Pa.) were tested on heparinized dog blood *in vitro* for their effects on a) sedimentation rate and on b) viscosity of blood and plasma at shear rates from 0.2 to 230 sec^{-1}. Determinations of viscosity were made at 37°C with both the Brookfield cone-plate viscometer (Brookfield Engineering Laboratories, Stoughton, Mass.) (3) and the Polarad couette viscometer (on temporary loan from the Polarad Electronics Corp., Long Island City, N. Y.)(4). Although the Brookfield instrument can be set for fairly low shear rates (down to 0.3 sec^{-1}) the torque with plasma or blood at these settings is so small and the readings so near zero on the scale, that they are essentially meaningless. Hence the Brookfield viscometer

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