is responsible for the abnormal permeability of the mitochondrial membrane and the altered microscopic appearance.

1. Pearce, G. W., *in* "Muscular Dystrophy in Man and Animals." (G. H. Bourne and M. N. Gorlarz, eds.), p. 159. Hafner, New York (1963).

2. Fisher, E. R., Cohn, R. E., and Danowski, T. S., Lab. Invest. **15**, 778 (1966).

3. Dillon, J. F. and March, B. E., Proc. Soc. Exptl. Biol. Med. **128**, 667 (1968).

4. Wrogeman, K. and Blanchaer, M. C., Can. J. Biochem. **45**, 1271 (1967).

5. Luft, R., Ikkos, D., Palmieri, G., Ernster, I., and Afzelius, B., J. Clin. Invest. **41**, 1776 (1962).

6. van Wijngaarden, G. K., Bethlehem, J., Meijer, A. E. F. H., Hülsmann, W. C., and Feltkamp, C. A., Brain **90**, 577 (1967).

7. Chappell, J. B. and Perry, S. V., Nature **173**, 1094 (1954).

8. Gornall, A. G., Bardawill, C. J., and David, M. M., J. Biol. Chem. **177**, 751 (1949).

9. Sugita, H., Okimoto, K., Ebashi, S., and Okinaka, S., *in* "Exploratory Concepts in Muscular Dystrophy" (A. T. Milhorat, ed.), p. 321. Excerpta Medica Foundation (1967).

10. Sreter, F. A., Ikemoto, N., and Gergely, J. *in* "Exploratory Concepts in Muscular Dystrophy" (A. T. Milhorat, ed.), p. 289, Excerpta Medica Foundation (1967).

11. Zymaris, M. C., Epstein, N., Saifer, A., Aronson, S. M., and Volk, B. W., Am. J. Physiol. **196**, 1093 (1959).

12. Vignos, P. J., Jr. and Warner, J. L., J. Lab. Clin. Med. **62**, 579 (1963).

13. Lehninger, A. L., "The Mitochondrion," p. 230. Benjamin, New York (1964).

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Gold Thiomalate–Induced Weight Gain in Guinea Pigs* (33372)

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Obesity can be induced in mice with a single dose of gold thioglucose (1). After injection, gold localizes in the neurones of the ventromedial area of the hypothalamus, presumably due to the special affinity of these cells for the glucose moiety (2–4). The animal becomes hyperphagic and obese when this hypothalamic area concerned with the regulation of food intake is damaged by the heavy metal. Gold compounds other than gold thioglucose are not effective (5, 6). Thus far, gold-induced obesity has been observed only in mice.

In a previous study of the effects of gold sodium thiomalate on the immune response of guinea pigs, treated animals were observed to gain weight at a faster rate than controls (7). This observation was investigated to determine whether the weight increase observed in gold thiomalate–treated guinea pigs was due to a mechanism comparable to that of gold thioglucose–induced obesity in mice.

Materials and Methods. Male Hartley strain guinea pigs were singly caged and given Purina chow and tap water *ad libitum*. Animals were randomly divided into two groups. Gold sodium thiomalate (Myochrysine, Merck) was administered intramuscularly to nine guinea pigs at a weekly dose of 2.1 mg. Ten control animals received a comparable dilution of sodium thiomalate in the same vehicle, but without gold. All animals received a weekly intramuscular injection and were handled identically throughout the experiment. The animals were weighed weekly and observed closely for any abnormalities. No toxic manifestations were observed in either group.

After nine weeks of treatment (ten injections), the animals were sacrificed and examined for retroperitoneal fat deposits. The omentum was carefully dissected, weighed, and repeatedly homogenized in a 2:1 chloroform to methanol mixture (8). The extracta-

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TABLE I. Effect of Gold Thiomalate on Weight Change of Guinea Pigs.[a]

Week	Cumulative mean weight gain[b] Gold-treated	Control	p
1	37 ± 7	46 ± 3	—
2	121 ± 8	124 ± 4	—
3	195 ± 10	194 ± 9	—
4	244 ± 15	237 ± 14	—
5	298 ± 15	284 ± 16	—
6	349 ± 17	328 ± 15	—
7	405 ± 19	378 ± 13	—
8	439 ± 20	405 ± 13	$<.10$ $>.05$
9	461 ± 20	410 ± 14	$<.025$ $>.01$

[a] Treated animals (initial mean weight of 9 treated guinea pigs = 342 g) received 2.1 mg gold sodium thiomalate i.m. weekly beginning one week prior to the first values listed. Controls (initial mean weight of 10 controls = 337 g) received sodium thiomalate without gold.

[b] Shown are means (g) ± 1 SEM.

ble lipid was dried and weighed.

Tissue sections from the thalamus and hypothalamus were weighed, individually fixed in Bouin's solution, and embedded in paraffin. The fixed sections from both control and gold-treated animals, with appropriate blanks and gold standards, were made radioactive by neutron bombardment at the Radiation Center of Oregon State University, Corvallis. Samples were exposed to a flux of 7 × 10^{11} neutrons/cm^2/sec for a period of 1 hr. After a decay period of 72 hr, emissions were counted in a gamma-ray scintillation counter according to the method of Debons and co-workers (3). Gold content was determined from a standard curve and expressed as micrograms of Au-198 per gram of tissue. After neutron-activation analysis for gold content, 7-μ sections were prepared. Autoradiography was performed at 18° for 21 days using an NTB 2 liquid emulsion. Sections were developed, stained with hematoxylin and eosin, and examined for precipitated silver grains and histologic abnormality.

Results. Gold thiomalate–treated guinea pigs and sodium thiomalate–treated controls initially showed identical weight gains. With continued weekly injections, however, it became apparent that the increase in weight of the gold-treated animals was greater than that observed in controls. The weekly cumulative mean weight change of the treated and control groups is shown in Table I. After the third week of gold injections, the rate of weight gain in the treated animals was greater than that of the controls. The difference in weight gain was significant at $p < .025$ at the ninth week. Significance of the results was estimated by Student's t test.

Throughout the experiment, food consumption (determined daily by weighing food remaining in the cages) of treated animals was not statistically different from amounts consumed by controls. Determinations of physical activity as measured in spin-wheel cages similarly did not show a significant difference between the two groups.

With the development of significant increased weight gain in treated guinea pigs after nine weeks of injections, all animals were sacrificed. Inspection of the carcasses revealed grossly increased retroperitoneal fat deposits in the gold-treated animals. The omenta were dissected, weighed, and studied for extractable fat. As shown in Table II, the mean omentum weight of gold-treated animals was significantly greater than that of the controls. Furthermore, larger amounts of fat could be extracted per 100 g body weight from omenta of treated animals. The difference between the two groups was significant at $p < .025$.

Sections from the brains of treated and control animals were processed for neutron-activation analysis. Samples were obtained from thalamic and hypothalamic regions (to

TABLE II. Omental Weight and Extractable Fat in Gold Thiomalate-Treated Guinea Pigs.[a]

Tissue	Gold-treated	Control
Omentum (wet)	3.4 ±.2	2.9 ±.2[b]
Omentum/100 g body wt.	.39 ±.01	.35 ±.01[c]
Extractable fat (dried)	.27 ±.02	.22 ±.02[c]
Extractable fat/100 g body wt.	.032±.001	.026±.001[b]

[a] Shown are mean values ± 1 SEM for 9 treated animals and 10 controls. All values are in grams.

[b] $p < .025$.

[c] $p < .05$.

include ventromedial areas), weighed, and activated along with appropriate blanks, controls, and gold standards. Gold was detected in the hypothalamic sections of treated animals at a concentration of 0.25 μg ^{198}Au g wet tissue, and in the thalamic sections at a concentration of 0.34 μg. Brain sections from control animals did not contain gold. Autoradiography of the activated brain sections revealed accumulation of silver grains within brain macrophages scattered throughout both thalamic and hypothalamic areas. Gold was not localized in specific areas. No lesions were observed in either the hypothalamus or thalamus of treated and control animals.

Discussion. The results indicate that guinea pigs receiving 2.1 mg gold sodium thiomalate per week gain weight more rapidly than control animals. No other toxic effects were observed at this dose level. The data obtained duplicate findings of a previous experiment (7) performed under identical conditions in which each of 10 guinea pigs (mean weight = 258 g) receiving 2.1 mg of gold sodium thiomalate per week gained an average of 561 g in 12 weeks, whereas 10 controls (mean weight = 256 g) gained 460 g per animal. The difference was significant at the $p < .0125$ level. Effects of this gold compound on females and on adult guinea pigs is not known since young males were used in both experiments.

The increased weight of the treated animals was found to be due, in part, to an increase in body fat as determined both by examination of retroperitoneal fat deposits and by quantitation of fat extracted from the omentum. Abnormalities of growth or bone structure and size were not detected.

Only gold thioglucose, not gold thiomalate, induced obesity in the mouse (1–6), due to the entrance of the gold thioglucose compound into the ventromedial neurones of the hypothalamus. The subsequent destruction of this area concerned with the regulation of food intake resulted in hyperphagia and obesity. Our finding of increased weight gain in a different species, the guinea pig, treated with a non–glucose-containing gold compound, gold thiomalate, prompted additional studies to determine whether the mechanism was comparable to that seen in gold thioglucose–obese mice. Sections of the hypothalamus were studied for accumulated gold by neutron activation and autoradiographic analyses. These procedures failed to localize the heavy metal within the ventromedial area. Lesions were not detected. The absence of hypothalamic localization of gold is in accord with previous observations that gold thiomalate fails to induce hypothalamic damage in mice or rats and does not consistently accumulate in specific brain areas (2, 3, 6).

The lack of ventromedial area gold localization and damage and the absence of hyperphagia in gold thiomalate–treated guinea pigs suggest the obesity is not the consequence of an altered food-regulating mechanism. Since gold compounds are known to inhibit numerous enzymes (10), it is possible the obesity observed in these experiments was secondary to an effect of gold thiomalate on fat metabolism. The site of the basic metabolic disturbance is, however, unknown.

The localization of gold in macrophages in brain tissue of treated guinea pigs is in agreement with previous studies showing that gold is concentrated in the organs of the reticuloendothelial system (9), and particularly within phagocytic cells (10). The relationship of this observation to the increased weight gain is not apparent.

Summary. Repeated injections of gold sodium thiomalate in guinea pigs resulted in significant weight gain. Retroperitoneal fat deposits and extractable omental fat were increased in treated animals. Neutron activation analysis and autoradiography localized gold within brain macrophages of treated animals. However, specific concentration of gold in hypothalamic nuclei and tissue destruction were not observed. The absence of lesions and the failure to demonstrate hyperphagia suggest that gold thiomalate induces a metabolic obesity rather than a regulatory obesity in guinea pigs.

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1. Brecher, G. and Waxler, S. H., Proc. Soc. Exptl. Biol. Med. 70, 498 (1949).
2. Marshall, N. B., Barrnett, R. J., and Mayer, J., Proc. Soc. Exptl. Biol. Med. 90, 240 (1955).
3. Debons, A. F., Silver, L., Cronkite, E. P., Johnson, H. A., Brecher, G., Tenzer, D., and Schwartz, I. L., Am. J. Physiol. 202, 743 (1962).
4. Brecher, G., Laqueur, G. L., Cronkite, E. P., Edelman, P. M., and Schwartz, I. L., J. Exptl. Med. 121, 395 (1965).
5. Marshall, N. B. and Mayer, J., Am. J. Physiol. 178, 271 (1954).
6. Mayer, J. and Marshall, N. B., Nature 178, 1399 (1956).
7. Persellin, R. H., Hess, E. V., and Ziff, M., Arthritis Rheumat. 10, 99 (1967).
8. Hanahan, D. J., "Lipide Chemistry," p. 11. Wiley, New York (1960).
9. Swartz, H. A., Christian, J. E., and Andrews, F. N., Am. J. Physiol. 199, 67 (1960).
10. Persellin, R. H. and Ziff, M., Arthritis Rheumat. 9, 57 (1966).

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Role of Phospholipids in Iodine Binding* (33373)

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Nonspecific binding between halides and phospholipids was first reported by Phillips *et al.* (1) who observed that most of the fluoride present in egg yolk was associated with acetone-insoluble lipids, after alcohol extraction. Christensen and Corley (2) later reported that a portion of bromide administered to animals, or added *in vitro* as sodium bromide to lipid solutions, could be recovered in the phospholipid fraction, and that this was true with sodium chloride or sodium iodide. Vilkki (3) demonstrated the presence in the thyroid of lecithin-like material that could reversibly bind iodide ion *in vitro*. Schneider and Wolff (4) further studied the problem to show that the iodide-concentrating effect was not merely one of nonspecific anion binding to a positively charged phospholipid species soluble in nonpolar solvents. These authors as well as Vilkki (5) have shown that the iodide-concentrating effect is not shared by synthetic dipalmitoyl, di-oleyl, or calf brain lecithin.

Our present interest in this subject was stimulated by a chance observation of high radioactivity in serum phospholipids isolated from a thyroid cancer patient given a therapeutic dose of $Na^{131}I$. Such a phospholipid-iodide complex formed *in vivo* has not been documented and the present study was undertaken to examine both *in vivo* and *in vitro* formation of a phospholipid-iodide complex.

Materials and Methods. In vivo *studies.* Albino rats weighing 200–300 g were given an oral dose of $Na^{131}I$ (50–60 μCi $Na^{131}I$, carrier-free) and sacrificed after 48 hr. Livers were perfused with 0.9% NaCl via the portal vein to remove blood from the liver tissue. They were then excised and washed thoroughly in tap water before extracting the lipids.

To study the incorporation of radioactivity in the thyroid, liver, and blood plasma, larger animals, such as rabbits, were used. Rabbits weighing 2.5–3.5 kg were given an intraperitoneal injection of $Na^{131}I$ (4–5 μCi) and sacrificed 24 hr later. Blood was collected by heart puncture, and the thyroid and

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