suggest that "moderate" and "heavy" exercise as distinguished in these experiments are physiologically as well as quantitatively, of different orders of magnitude. Similarly, in the mouse experiments there is no indication of any effect, beneficial or deleterious, of heavy early swimming, as opposed to the indicated beneficial influence of moderate exercise.

Swimming seems a justifiable test of the effects of more natural types of exertion. The control experiments on mice show that the indifferent role of swimming or the perhaps beneficial effect of moderate early swimming does not represent an overcompensation of harmful exercise by a protective action of immersion in tepid water. If anything, swimming, as already indicated, may be responsible for the apparent ill effect of heavy "exercise" in dogs judged by the significantly greater pulmonary edema of heavy swimmers as opposed to controls. Furthermore, it may be valid to assume that, from the point of view of muscular, circulatory and respiratory mechanisms, swimming is at least as vigorous an activity as that envisaged for the soldier gassed at equivalent levels; we have here subjected exertion to a perhaps unduly rigorous test.

Finally, it may be pointed out that exercise by a diphosgene patient or other pulmonary irritant patient during the clinical latent period need not be expected *a priori* to be adding insult to injury. The water and blood demands of muscle during exercise could tend to delay the onset of edema. Once edema and attendant anoxaemia set in, however, increased oxygen demand becomes an undesirable feature of exercise. Since the time of onset of edema is variable, depending mainly on severity of exposure, which is in turn a function of more than just gas concentration and exposure duration, no particular time can be set as the limit of a safe period.

15627 P

Inositol Decholesterization in Old Hens.*†

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Inositol was found by Gavin and McHenry[1] to prevent the development of a special type of biotin fatty liver, but no such lipotropic activity was demonstrated against thiamin fatty livers. Abels *et al.*[2] found that inositol accounted for the lipotropic property of a lipocaic preparation when given to patients with gastrointestinal cancer. Inositol administered preoperatively, reduced the average concentration of fat to 46% of that of almost uniformly fatty infiltrated livers of untreated patients. Shay[3] could demonstrate no reduction in the size of fatty livers in diabetics nor any changes in the blood cholesterol by the daily administration of 1.2 g of inositol. However, Russakoff and Blumberg[4] reported reduction in the blood cholesterol and cure by inositol in doses of 1 g per day, in a patient with a dermatological condition associated with a disturbed fat metabolism.

* With the technical assistance of Anna H. Williams, Lucy Prosise, H. Tom Leigh, John Prewett, and George W. Reimer.

† Inositol was generously supplied and the study was, in part, supported by a grant from the Medical Research Department of the Winthrop Chemical Co.

1 Gavin, G., and McHenry, W. E., *J. Biol. Chem.*, 1941, **139**, 485; 1943, **148**, 25.

2 Abels, J. C., Kupel, C. W., Pack, C. T., and Rhoads, C. P., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 157.

3 Shay, H., *Am. J. Digest. Dis.*, 1943, **10**, 48.

4 Blumberg, H., and Russakoff, A. H., unpublished data, quoted in *Ann. Int. Med.*, 1944, **21**, 848.

TABLE I.
Effect of Inositol on Blood and Tissue Lipids.

	Blood cholesterol, mg/100 ml		Phospho-lipids	Tissue Cholesterol, mg/100 g					
				Aorta		Heart		Liver	
	Total	Esters	mg/100 ml	Total	Esters	Total	Esters	Total	Esters
Controls	274.8	166	9.6	230	176.2	275	223	340	240
36 old hens S.D.	±39	±19	±2.7	±41	±20.4	±71	±19	±65	±24
(A) Series (7)									
Inositol 1st Bl.*	254 ±28	201±29	7.5±1.7						
25-30 2nd Bl.	220 ±29	169±26	10 ±1.1						
days 3rd Bl.	202 ±18	151±16	9.4±0.7	210	160	172	126	281	221
				±10	±19	±9.6	±9	±28.5	±23.5
(B) Series (16)									
Ino.† control									
1st Bl.	230 ±29	181±27	8.6±1.3						
Ino. 25 days									
2nd Bl.	206 ±20	160±20	11.3±1.9						
Ino. 55 to 68 days				197	145	167	127	241	178
3rd bl.	176 ±13	140±12	12.9±1.9	±27	±22	±40	±29	±60	±35
(C) Series (23)									
Inositol 1st Bl.	237 ±29	187±31	8.7±1,2						
2nd Bl.	210 ±24	163±20	11 ±1.9						
30-68 days 3rd Bl.	184 ±19	135±16	11.8±1.9	201	155	166	129	253	191
				±38	±24	±37	±29	±55	±93

* Bl.—Bleeding. †Inositol. S.D.—Standard Deviation, ±.

Further studies seemed to be in order and inositol was therefore subjected to animal experimental studies similar to those made with other lipotropic substances.[5]

Two series of old hens from high egg-producing stock were individually caged and given the standard high-fat laying mash (Purina). Blood was withdrawn from 16, and later 7 more, hens for the determination of control levels of total cholesterol, cholesterol esters, total and inorganic phosphorous levels by the methods of Bloor,[6] Bloor and Knudson,[7] and King,[8] respectively.

Inositol, 0.5 g was then added to the diet, and put down the gullet of each hen daily. All the birds were healthy throughout the experiment. After 25 days blood was again removed for the chemical studies and at the sacrifice after 30 days in 7 hens and after 62 days in 16 hens. The aorta and approximately 0.25 g of heart muscle and a similar mass of liver were removed, weighed accurately and macerated with sand and extracted with ether alcohol. The 10 cc extract of each organ was analyzed and the amount of total cholesterol and cholesterol esters in 100 g of each tissue calculated. The statistically-treated data for the 7 hens treated for 25 to 30 days and for the 16 hens treated for 55 to 68 days are set down in Table I and for all 23 inositol-treated hens for comparison with the normals previously established.[5]

It is evident that barely significant reductions in cholesterol in blood and tissues develop in 30 days while definite reductions have been recorded in 55 to 68 days.

Under the conditions of this experiment, the total blood cholesterol dropped about 11% and the cholesterol esters about 13%, but the organic or lipid phosphorous rose about 31% after 25 days of inositol. After 55 to 68 days, the average total blood cholesterol had dropped 22%, the esters 17%, and the phospholipids had risen 50%. The

[5] Herrmann, G. R., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 229 and 302. (Note: In these two communications the subheading under Tissue Cholesterol total/esters determinations on aorta, heart, and liver were erroneously set down as mg/250 mg instead of mg/100 g to which amounts the calculations had beeen made.)

[6] Bloor, W. R., *J. Biol. Chem.*, 1916, **24**, 227.

[7] Bloor, W. R., and Knudson, A., *J. Biol. Chem.*, 1916, **27**, 107.

[8] King, E. J., *Biol. Chem.*, 1932, **26**, 240.

tissue analysis of inositol-treated hens showed reductions in concentration of 9% and 9% of cholesterol total and esters in the aorta in 30 days and 14% and 12% in 52 to 68 days. The heart muscle levels dropped 37.5% and 43% in 30 days and 40% and 41% in 55 to 68 days, and those in the liver were 17.6% and 18.3% in 30 days and 25% and 20% in 55 to 68 days respectively below the levels in the controls established in previous studies.[8]

The results seem to indicate that inositol is a decholesterizing agent and effects some mobilization of cholesterol and cholesterol esters from the tissues.

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Prolongation of Penicillin Activity with Penicillinase-Inhibiting Compounds.

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Methods for prolonging the activity of penicillin in the body by use of various compounds to delay absorption or excretion have been proposed and reviewed fully in the literature. We have made extensive studies on an oral penicillin preparation composed of alum-precipitated penicillin or aluminum penicillin mixed with sodium benzoate. It has been determined from laboratory and clinical experience that sodium benzoate prolongs the blood serum levels when given with penicillin, either orally or parenterally. Other investigators studying this or similar compounds have explained the prolonged blood serum levels on the basis of renal blockage due to absorption of hippuric acid, formed from such compounds, by the renal tubules. Evidence to support this explanation is unconvincing with the dosage we have used. The literature is not clear as to how much sodium benzoate or benzoic acid is needed to produce sufficient hippuric acid to cause an effective renal blockage. In order to find another explanation we have attempted to demonstrate a mechanism that prevents or delays the destruction of penicillin by enzymes.

Normally the intestine contains a large number of "coliform" organisms that are known to produce penicillinase. If this is true then destruction of penicillin in the intestine might be prevented by (1) inhibition of the penicillinase-producing bacteria, or (2) allowing the bacteria to grow while preventing production of the enzyme, or (3) the destruction or inactivation of penicillinase produced.

The following preliminary experiments were made to clarify these points. To begin with, compounds such as sodium sulfathalidine and bromsaligenin, effective in inhibiting or killing the Gram-negative intestinal bacteria, were used. Substances having no inhibitory effect on the Gram-negative bacilli, such as sodium benzoate, sodium sulfanilate and *p*-amino benzoic acid, were selected for study as representing this group of compounds.

In the *in vitro* experiments flasks containing 100 ml of penicillin assay broth, F.D.A. modification, with 10 units of penicillin sodium (Lilly) per ml were inoculated with 0.2 ml of an 18-hour broth culture of *Escherichia coli*. Drugs were added to individual flasks in concentrations dependent upon their solubilities and estimated strength necessary to produce satisfactory results. Control flasks containing the drugs without penicillin were inoculated, and control flasks of penicillin with and without *Esch. coli* were incubated. Another control series in which the broth contained the penicillin and drug was inoculated with coli and incubated.

All flasks were incubated at 37°C. After