TABLE I. Effect of Estradiol on Liver Glycogen in Intact Rats and in Adrenalectomized Rats Treated with Adrenal Cortex Extract. Fifteen rats/group.

ACE, cc/day	Adrenalectomy Liver glycogen, mg/rat No estrogen	Adrenalectomy Liver glycogen, mg/rat Estrogen	Adrenals intact, no ACE Liver glycogen, mg/rat No estrogen	Adrenals intact, no ACE Liver glycogen, mg/rat Estrogen
1	9.5 ± .52	12.3 ± .21	16.6 ± 2.11	105.3 ± 8.30
2	8.7 ± .71	35.0 ± 3.27	20.5 ± 2.97	76.6 ± 6.23
4	9.5 ± .95	39.7 ± 3.07	16.6 ± 1.88	89.0 ± 6.41
5	13.4 ± .76	51.1 ± 4.44	18.2 ± 1.76	72.3 ± 5.08
6	28.4 ± 4.37	62.4 ± 5.11	19.7 ± 2.31	93.8 ± 7.20

enhances deposition of liver glycogen: response to estrogen was greater in the presence of adrenals. It is also possible that estrogen has no glycogenic effect of its own but causes a rise in titer of cortical hormones by suppressing rate of destruction of these steroids. Mills(5) has published evidence that such can occur.

The principal effect of the cortical hormones in supporting the glycogenic effect of estrogen may be a permissive one. The term "permissive" was first used to call attention to biological responses which fail to become overt in the adrenally insufficient animal but do occur in adrenalectomized animals maintained in a state of eucorticalism by a steady intake of cortical hormones. It is assumed that the permissive role of a hormone represents normalizing the functions of organs—sustaining the integrity of metabolic processes and thereby the capacity to adapt and respond. A large number of biological responses are now known to require the presence of cortical hormones but not the adrenal glands. The term "permissive," although still useful, covers our ignorance of several mechanisms by which hormones and other biological principles support homeostasis and adaptability.

Summary. Among 300 force-fed male rats of the Sprague-Dawley strain (300 g initial wt.), estrogen increased liver glycogen in the fasting adrenalectomized rat treated with ACE whereas this effect was not observed in the adrenally insufficient animal. The glycogenic effect of estrogen was greater in intact rats than in adrenalectomized rats treated with ACE.

Acknowledgement is made of the technical assistance of Mr. Mitchell Heller and Mr. Melvin Vinik on some experiments.

1. Long, C. N. H., *Endocrinology,* 1942, v30, 870.
2. Janes, R. G., Nelson, W. O., *Am. J. Physiol.,* 1942, v137, 557.
3. Ingle, D. J., Altamero, T. L., Jr., Flores, V., *Cancer Research,* 1956, v16, 437.
4. Peterson, R., Rose, D., *Canad. J. Technol.,* 1951, v29, 317.
5. Mills, Ivor H., Bartter, F. C., *Lancet,* 1958, v11, 941.

Received January 29, 1959. P.S.E.B.M., 1959, v100.

Effect of Dietary Iron on Phosphate Metabolism.* (24655)

WOLFGANG BUTTNER† AND JOSEPH C. MUHLER
Dept. of Biochemistry, Indiana University Medical Center, Indianapolis

In experiments involving the effect of different carbohydrates on the calcification process, differences were observed that could not be accounted for by any readily known mechanism. Upon further scrutiny of the dietary components as well as the experimental procedures, it was found that the mineral supplement used in these studies had an unusually large iron content. This observation prompted us to design a study in which the amount of

* This study was supported in part by grant from Procter and Gamble Co., Cincinnati, O.

† Post-doctorate fellow. Present address: Univ. of Mainz, Germany.

TABLE I. Effect of Extra Dietary Iron and Phosphate on Calcium and Phosphorus Content of Newly Formed Dentin of Rat Incisors and of Iron Content of Liver.

	Ca and P content of apical dentin in incisor teeth			Fe in livers	
Group	Ca, mg %	P, mg %	Ca:P	Total, mg	Conc., mg %
I	26.9	13.5	1.99	.9 ± .1*	38 ± 7
II	27.6	13.7	2.01	5.9 ± .5	210 ± 40
III	28.0	14.4	1.94	2.9 ± .3	120 ± 20
IV	23.8	12.5	1.90	2.8 ± .3	100 ± 10
V	26.7	13.8	1.93	.6 ± .1	25 ± 3
VI	25.6	13.8	1.86	.9 ± .2	50 ± 8

I = control; II = 50 mg Fe; III = 50 mg Fe + 50 mg P; IV = 50 mg Fe + 100 mg P; V = 50 mg P; VI = 100 mg P.

* Stand. dev.

phosphate in calcified tissues was evaluated when extra dietary levels of iron were administered to rats.

Experimental. A total of 48 male Sprague-Dawley strain rats, weighing between 92 and 108 g, were divided equally into 6 experimental groups according to their body weight. All animals received a stock corn diet and redistilled water *ad lib.*, the calcium and phosphorus content of which was 0.32 and 0.31 mg % respectively. The animals in Group I received only this diet. Group II animals received 50 mg Fe, those in Group III 50 mg Fe plus 50 mg P, and those in Group IV 50 mg Fe plus 100 mg P. Animals composing Groups V and VI received 50 and 100 mg P, respectively. Fe was administered as $FeSo_4$ and P as NaH_2Po_4, and both were given simultaneously by stomach tube daily throughout a 3 week period. After termination of the study period, the animals were sacrificed and the livers were removed and analyzed for iron content. The maxillary central incisors were also removed and the newly formed dentin removed and analyzed for calcium and phosphorus. All animals were housed separately in raised screen cages in an air-conditioned room.

Results. The data obtained in this study are found in Table I. Calcium content of the incisor dentin is higher in the group receiving Fe or Fe plus 50 mg P, but significantly lower ($p = .02$) when Fe plus 100 mg P is administered. When 50 mg P and no Fe is given, calcium content of incisor dentin is not affected, but when 100 mg P is administered there is less calcium in the dentin ($p = .06$). Phosphorus content of incisor dentin does not seem to be nearly as variable as calcium content and is higher when Fe plus 50 mg P is administered ($p = 0.09$) and significantly less when Fe plus 100 mg P is given ($p = 0.05$).

Iron content of liver shows the effect of extra dietary iron therapy. There is a 6 fold increase in iron content of livers of the group receiving 50 mg Fe when compared to controls. However, when 50 mg Fe plus 50 mg P is administered there is a highy significant ($p = 0.0001$) reduction in total liver iron content when compared to the group receiving the same amount of Fe but no added phosphate. Similar findings result when 50 mg Fe plus 100 mg P is given. No significant differences in liver iron content result when P is administered in absence of extra dietary iron.

Discussion. Increasing the dietary iron content significantly increases iron content of the liver. However, as the phosphate content of the diet is similarly increased, liver iron content is reduced, apparently by forming an insoluble iron-phosphate complex which decreases the mutual metabolic availability of both iron and phosphorus. These results are similar to those of Hegsted, Finch and Kinney (1) who reported that the amount of iron deposited in the liver of rats is inversely related to phosphorus content of the diet. Of considerable interest, however, are the results obtained by the incisor dentin analysis in relation to extra dietary iron and phosphorus content. When 100 mg phosphate was administered in the presence of iron, a significant reduction in incisor dentin calcium and phosphorus content was found. Apparently, the concentration of iron and phosphate at this level is sufficient to modify significantly the blood phosphorus content to cause a reduction in concentration of both calcium and phosphorus in the growing dentin. An analysis of blood phosphorus content of these animals corroborates this finding in that a 20% reduction in blood phosphorus level was found when compared to control animals. These data would suggest that more study be given to those factors which complex both dietary iron and phosphate and which affect their absorp-

tion from the GI tract in relation to their effect on the calcification process in general.

Conclusions. Increasing the amount of iron by means of stomach tubing $FeSo_4$ to rats significantly increases liver iron content. When 50 mg phosphate is given simultaneously with iron, a significant reduction in liver iron content results, as well as a significant diminution in calcium and phosphorus content of newly forming incisor dentin. These results, compared with a significant reduction in blood phosphorus concentration, suggest that increasing the iron content of the diet for prolonged periods of time without significantly increasing the dietary phosphorus content may result in improperly calcified tissues.

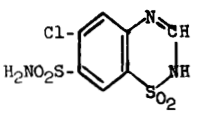
1. Hegsted, D. M., Finch, C. A., Kinney, J. D., *J. Exp. Med.* 1949, v90, 147.

Received January 9, 1959. P.S.E.B.M., 1959, v100.

Saluretic Activity of Hydrochlorothiazide (6-Chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide) in the Dog. (24656)

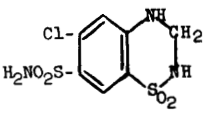
JOHN E. BAER, HORACE F. RUSSO AND KARL H. BEYER
Merck Inst. for Therapeutic Research, West Point, Pa.

Chlorothiazide,* first synthesized by Novello and Sprague(1), was shown to be a remarkably safe and potent agent for increasing excretion of sodium and chloride in animals (2,3) and in man(4). The agent has found widespread use in treatment of congestive failure, edema of pregnancy, premenstrual tension and other edematous states, and in management of hypertension. During research leading to discovery of the effectiveness of chlorothiazide as a saluretic-diuretic agent, many compounds more or less closely related to it in structure have been synthesized by Novello and Sprague and have been evaluated in these laboratories. We wish to report the preclinical evaluation of one such compound, hydrochlorothiazide,† that resembles chlorothiazide qualitatively but is considerably more potent. The structural formulae for the 2 compounds presented below make it evident that the new agent differs from chlorothiazide only in saturation of the heterocyclic portion of the molecule.



6-Chloro-7-sulfamyl-1,2,4-benzothiazine-1,1-dioxide

Chlorothiazide
Diuril*



6-Chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide

Hydrochlorothiazide
HydroDiuril†

Methods. Renal clearance studies were performed on trained unanesthetized female mongrel dogs in post-absorptive state. Water (500 ml by gavage) and isotonic mannitol-phosphate infusion at 3 ml/min. throughout experiment, assured adequate urine flow. Hydrochlorothiazide was introduced into the infusion during the "drug phase" of experiments at dosages indicated. Clearance of exogenous creatinine, administered subcutaneously, was used as a measure of glomerular filtration rate. Blood was collected at midpoint of each 10-minute clearance period. The powdered drug was given to dogs orally in gelatin capsules containing the solid compound. Fasting female mongrel dogs were intubated with 500 ml of water and urinary bladders were emptied by catheter. The animals were held in metabolism cages, and complete urine collections were made at regular intervals up to 6 hours. Hydrochlorothiazide was hydrolyzed in neutral solution (15 lb pressure for ½ hour) to a substance that can be measured by diazotization and coupling

* DIURIL is trade-mark of Merck & Co. for its brand of 6-chloro-7-sulfamyl-1,2,4-benzothiadiazine-1,1-dioxide, the nonproprietary name of which is chlorothiazide.

† HYDRODIURIL is trade-mark of Merck & Co., for its brand of 6-chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide, the nonproprietary name of which is hydrochlorothiazide.