hibitory factor is separate and distinct from the pyrogenic factor in the extracts of normal male urine. Until such time as the gastric inhibitory factor is definitely identified, we propose to call it urogastrone.

This work is reported at this time in preliminary form because of its significance for other fields of endocrinology. It is well known that many extracts of various organs are unsuitable for intravenous administration because of fever reactions. We have assayed several commercial extracts of urine and found them to contain pyrogen. Pyrogenic substances may, in fact, be expected to occur in all extracts of biological origin which have not been treated in such a way as either to remove it or to prevent its development.[4] As long as there is no relationship between the effects of pyrogen and those of the specific component of an extract, the presence of pyrogen presents merely a problem of purification. However, when the properties of an extract resemble those of pyrogen, one should be cautious in assuming the presence of a specific component until the absence of pyrogen has been demonstrated. In view of this fact, the testing of extracts of the specific metabolic principle of the pituitary and of the antidiuretic principle of urine for pyrogen would seem to be desirable.

Conclusions. 1. Extracts of normal male urine prepared by the benzoic acid adsorption method of Katzman and Doisy contain large amounts of a pyrogenic substance, as well as the gastric inhibitory factor, urogastrone. 2. Methods for the preparaation of pyrogen-free urogastrone have been developed.

11153

Concentration and Detection of a Dye in Abscesses.

H. KROLL, SIEGFRIED F. STRAUSS AND H. NECHELES.

From the Departments of Gastro-Intestinal Research of Michael Reese Hospital, and of Physiology, University of Chicago, Chicago.

In a previous communication from this laboratory by Strauss, Neuwelt, Rovner and Necheles[1] a method was described for the detection of hidden abscesses and carcinomas. This method was based on the fact that certain dyes and colloidal substances are concentrated

[4] Ivy, A. C., and Gray, J. S., *Int. Abstr. Surg.*, 1939, **69**, 409.

[1] Strauss, S. F., Neuwelt, F., Rovner, L., and Necheles, H., *Surgery*, 1938, **4**, 930.

more or less selectively in abscesses and carcinomas. Our plan was to employ compounds which emit gamma rays and to detect the focus of their greatest concentration in the body, *i. e.,* the abscess or carcinoma, by the localization of the greatest concentration of radiation leaving the body. A sensitive Geiger-Müller counter was used and described. In experiments on dogs colloidal thorium dioxide was used as source of gamma rays and we were able to detect a high percentage of abscesses in these animals. It was realized, of course, that in the human this compound cannot be employed, because of the dangers involved.[2] It was planned, therefore, to use substances with only temporary radioactivity. In the light of our present knowledge a half-life period of radioactivity of even several days cannot be considered dangerous if sufficiently small quantities of the substance are used. Before trying out such radioactive compounds on animal or man, it was necessary to do preliminary work on their synthesis, toxicity and local concentration and the results of this work are reported here. Of the elements which are capable of being radioactivated, bromine appeared to be most promising because of its favorable half-life period, and because the reactivity of the free bromine lends itself to the synthesis of a compound which will act as a suitable carrier for the radioactive halogen. Improvements in the procedure for the concentration of the radioactive element have made it readily available in sufficient quantities for synthetic purposes.[3]

Recent investigations have shown conclusively that certain vital dyes will localize in malignant tumors and inflamed tissue. A bisazo, acid dye, T1824, which has been used most frequently and successfully,[4, 5] proved to be of special interest. This dye possesses 2 active naphthalene radicals which are capable of undergoing a chemical reaction with bromine and thereby providing a means of introducing the radioactive atom into the body in a non-toxic form which may be concentrated in the desired area. The presence of amino and hydroxyl groups in the 1,8 positions of the naphthalene nucleus activates the positions ortho and para to these groups, enabling the dye molecule to pick up one, 2, 3, or 4 atoms very readily from an aqueous bromine solution.

A quantitative investigation of the localization process was necessary in order to determine which of the Br-dye compounds offered

2 Laborde, S., *Presse Médicale,* 1936, **44**, 1915; Taft, R. B., *Radiology,* 1937, **29**, 530.

3 Lu, C. S., and Ludgen, S., *J. Chem. Soc.,* 1939, 1219.

4 Duran-Reynals, F., *Am. J. Cancer,* 1939, **35**, 98.

5 Menkin, V., *J. Exp. Med.,* 1932, **56**, 157.

optimum results, *i. e.*, highest concentrations in abscesses and lower concentrations in organs and tissues.

Although the literature presents ample qualitative studies on the concentrations of dyes in tissues and organs, there has been relatively little work of a quantitative nature; most investigators depending on the staining properties of the dye as a basis for determinations of the relative amounts of dye adsorbed. This means of analysis offers a serious objection in that the natural colors of the tissues and organs seriously interfere with even a visual approximation of the concentration of dye adsorbed. The addition of a bromine atom to the dye molecule permits a quantitative investigation, by the use of a suitable chemical analysis, of the actual amount of dye adsorbed per unit amount of tissue.

Experiments and Results. Preparation of dye solution. One hundred milligrams of T1824 (Evan's Blue) was dissolved in 15 ml of water. A given volume of an aqueous bromine solution, which had been previously analyzed, was added dropwise slowly, with continual stirring until the theoretical amount of bromine had been taken up.

The reaction proceeded smoothly (at room temperature) with a shifting of the color of the solution from blue to purple and finally to red-brown. If an amount of bromine greater than four equivalents was added to one equivalent of dye, precipitation of the dye took place. A sufficient amount of $NaHCO_3$ was added to neutralize the hydrogen bromide formed during the reaction and to assure a proper pH for intravenous injection of the dye solution.

Injection of Dye Solution. A suspension of streptococci and infusorial earth,* or an aqueous suspension of dogs' feces was injected subcutaneously into the right thigh of a dog. The abscess was allowed to develop for 24 hours. The dye solution, freshly prepared, was injected intravenously. In no case, did the dog exhibit any toxic effects from small doses of the dye (0.1 g). However, with much larger doses, the dye compounds with 2 and 4 Br atoms were toxic, in some cases causing death of the animal.

Analysis: The animal was sacrificed 20 hours after the injection of the dye. Samples of blood, abscess tissue, corresponding healthy tissue, spleen, kidney, liver and duodenum were taken. Examination of the internal organs showed staining of the gastrointestinal tract, spleen, and liver. The intensity of the staining decreased with the amount of bromine introduced into the dye molecule. With the

* Suggested by Dr. W. Bloom of the Department of Anatomy, the University of Chicago.

monobrominated dye, the staining of the region of the abscess was apparent in a few hours.

The wet samples were dried overnight in an oven at 110°C. The method used for the analysis was that of Brodie and Friedman.[6] One-half to one gram samples of dried tissue were fused with NaOH in a nickel crucible. Potassium nitrate crystals were carefully added until complete oxidation of the fused organic matter was assured. The solid melt was taken up in distilled water and neutralized with concentrated sulfuric acid. The bromine, present as bromide, was oxidized to bromate by the action of sodium hypochlorite in the presence of sodium dihydrogen phosphate. The bromine concentration was then determined by the iodometric titration of the bromate present.

Results. Table I tabulates the number of milligrams of bromine found per gram of dried tissue with the various dye derivatives. It is of interest to note that with the tetra and di-substituted dye, although the amount of bromine found in the abscess was double that found in the control, spleen, liver and kidney contained an amount of bromine which was roughly twice that found in the abscess. The amount of the mono-substituted dye concentrated in abscess, liver, kidney and spleen is of the same degree of magnitude. This fact is brought out much more effectively by Table II. Selecting

TABLE I.
Concentration of Brominated Dye in Tissues as Determined by Their Bromine Contents.

	Abscess	Control	Liver	Kidney	Spleen	Intestine
A. 4 atoms of bromine per molecule of T1824. Values in mg Br/g dried tissue.						
	.0525	.0277	.0881		.117	.0260
	.0555	.0393	.0846	.115	.0898	.0458
	.0552		.151	.0886	.136	.0390
	.0616	.0144	.101		.0918	.0482
	.0682					
Avg	.0566	.0271	.106	.102	.109	.0397
B. 2 atoms of bromine per mol. of T1824.						
	.0452	.0203		.0670	.110	.0450
	.0377	.0266	.0858	.0993	.123	.0436
		.0222	.0963	.0995	.103	.0281
	.0491					
Avg	.0440	.0230	.0910	.0886	.112	.0389
C. 1 atom of bromine per mol. of T1824.						
	.0645	.0269	.0645	.0760	.0701	.0347
	.0703	.0321	.0439	.0690	.0505	.0413
	.0734					
Avg	.0694	.0295	.0542	.0725	.0603	.0380

[6] Brodie, B. B., and Friedman, M. M., *J. Biol. Chem.*, 1938, **124**, 511.

TABLE II.

Calculated Concentration of Brominated Dyes.

Ratio of dye* concentrated in:	2 Br / 4 Br		1 Br / 4 Br	
Abscess		= 1.57		= 4.92
Control	"	1.69	"	4.34
Liver	"	1.72	"	2.04
Kidney	"	1.77	"	2.90
Spleen	"	2.06	"	2.22
Intestine	"	1.95	"	3.84

* The relative concentration of the mono-, di-, and tetra- substituted compounds in the tissue was found by dividing the amounts of Br obtained in the analysis by 1, 2, and 4 respectively.

the tetrabrominated molecule as a standard, column one of Table II shows that nearly twice as much of the di-substituted derivative is adsorbed in the abscess as compared to the tetra-substituted one. This means that of the 3 brominated dyes the one containing 4 Br atoms showed the least concentration in the abscess.

Column 2 of Table II illustrates the corresponding ratios between the mono- and tetra-substituted compounds. The ratio approximates 5 for the abscess, and 4 for control and intestine. Liver, kidney and spleen show a decided drop, the ratio indicating only twice as much concentration of the mono-brominated dye. It is apparent, therefore, that the concentration in the abscess of the monobrominated dye is 5 times that of the tetrabrominated one.

Analysis of the blood showed that in no case was the amount of bromine present in 1 cc of blood greater than that found in one gram of control tissue. In most instances, the amount present was smaller.

In the preparation of the dye an equivalent amount of hydrogen bromide was liberated. Recent work[7] has shown that bromide ion injected into a normal animal is present in the extra-cellular fluid, and does not concentrate preferentially in any given region. It can be shown that the small amount of bromide ions thus introduced when brominated dye is injected, is distributed over the entire animal body and will produce only a slight error in our analysis which is well within the range of the maximum probable analytical error.

Discussion. An investigation of the permeability of a cell membrane to a substance must involve two considerations (1) the nature of the cell membrane, and (2) the nature of the substance. Menken[5] has shown that inflammation produced by foreign substances is characterized by certain processes; besides other changes, increased permeability and a higher acidity in the region of inflammation is

7 Weir, E. G., and Hastings, A. B., *J. Biol. Chem.*, 1939, **129**, 547.

observed. Duran-Reynals[4] in his studies on dye localization in tumors, has found increased permeability of certain tumor tissues to foreign proteins and dyes. The presence of a high lactic acid concentration in malignant growths has been described by Warburg.[8] The occurrence of an increased permeability and acid reaction in inflamed and tumor tissue is significant and offers a probable clue as to the mechanism of dye absorption in these tissues.

The dye T1824, in an aqueous solution is a colloid which is negatively charged because of the ionized sulfonic acid groups in the molecule. The difference in polarity between the dye molecule and the inflamed tissue will cause the dye particle to be adsorbed on the surface of the cell membrane. The amount of dye adsorbed will depend upon its colloidal size, and the permeability of the capillary wall. Therefore, other factors being constant, more of the monobrominated dye will be adsorbed than of the tetrabrominated derivative.

The retention of dye by cells may take place by a second process, that of solution of the dye in the lipoid material of the cell wall. Höber[9] has shown that symmetrical disulfonated mono-azo dyes pass through a perfused kidney, whereas unsymmetrical disulfonated dyes are retained.

The presence of a bromine atom in a dye molecule will increase the hydrophobic as well as the lipophilic nature of the dye. It is to be expected that the tetrabrominated molecule will exhibit the maximum solubility in material of a lipoid nature. In normal organs, the adsorption of a dye due to an electrostatic effect may be assumed to be constant, and the amount of dye taken up will be a function of the concentration of the dye and its lipoid solubility. This will then explain the decrease in the ratio of dye retention in Table II, column 2, for liver, kidney and spleen. This property of the monobrominated dye makes it the most suited one for our purpose of the 3 brominated compounds studied. Modifications of experimental conditions indicate that the amount of dye localized in abscess, spleen, kidney, and liver may be influenced. These modifications are being investigated in order to achieve a maximum concentration of dye in tumor or abscess tissue and a minimum in other organs.

Summary. In continuation of our work on the detection of hidden

8 See Burk, Dean, On the Biochemical Significance of the Pasteur Reaction and Meyerhof Cycle in Intermediate Carbohydrate Metabolism. Page 121 of Some Fundamental Aspects of the Cancer Problem, Supplement of *Science*, Vol. 85, No. 4, New York, June, 1937.

9 Höber, R., and Briscoe, P. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **41**, 624.

abscesses, a study of the localization of brominated derivatives of the dye T1824, in abscess tissue and organs was made. The monobrominated dye was found to be best suited for future study. It was concentrated to a much lesser degree in liver, kidney and spleen and to a somewhat higher degree in the abscess than the di- and tetrabrominated compounds. The mechanism for the localization of the dye derivatives based on physico-chemical processes is discussed.

11154 P

Histological Demonstration of Vitamin A in the Human Liver by Means of Fluorescence Microscopy.

HANS POPPER.* (Introduced by A. C. Ivy.)

From the Cook County Graduate School of Medicine and the Cook County Hospital, Chicago, Ill.

It has been shown[1] that in liver and adrenal of rats there is a green fluorescence which fades during irradiation. This was attributed to presence of Vitamin A. Bodies showing morphologically similar green fading fluorescence were visualized in human organs. They gave histochemical reactions identical with those observed in normal rats or in Vitamin A deficient rats realimented with Vitamin A. These reactions are: solubility in alcohol, acetone, and mineral oil, and insolubility in water, acids, alkalis, glycerin, and reducing agents. The green fluorescence was not visible after treatment with oxidizing agents or prolonged fixation, especially at temperatures above 20°C.

The characteristic fluorescence, attributed to the presence of Vitamin A, was studied in 113 human livers by means of fluorescence microscopy. There was great variation in amount and distribution of the green fluorescing bodies. This observation agrees with chemical determinations made by Moore[2] and others. Usually the Kupffer cells showed small fluorescing droplets. After staining with phosphin 3 R they revealed silver-gray fluorescence. This was also found if staining was applied after fading of the green fluorescence. This indicated the presence of a carrier substance of lipoid character. The silver-gray fluorescence was absent after acetone treatment. In in-

* Research fellow of the Cook County Graduate School of Medicine.

1 Popper, H., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 133.

2 Moore, T., *Biochem. J.*, 1937, **31**, 155.