THE INFLUENCE OF URIC ACID ON THE PERMEABILITY OF MEMBRANES

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PLATE 1

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During investigations concerning the action of uric acid upon the tissues, and especially upon vital staining of them with trypan blue (1), the question arose of whether uric acid has an influence on the permeability of cellular membranes. The relatively pronounced staining noticed in animals treated with uric acid and with trypan blue did not provide the answer to this question, since the uric acid brought about alterations of the organs eliminating the dye (kidneys). It seemed probable that the obstacle to elimination resulted in a relatively great concentration of the dye in the blood and an unusual partition of the dye between the plasma and the tissues, according to an equilibrium proved by Smith (2). Dyes have since been used which are only slightly eliminated through the kidneys or not at all, for example, Congo red or vital red (Dawson, Evans and Whipple (3)). Treatment with uric acid can give rise to alterations also of the liver when it is given in large doses or for a long time; and the liver is the probable organ of elimination for Congo red (personal researches). We have tried to avoid the complicating factor by the use of small doses only.

For the study of the biological action of uric acid, rats were chosen as especially suitable on account of their peculiar purin metabolism (4). The following phenomena were noted.

1. Rapid and complete absorption of the dye from the peritoneal cavity within 24 hours.

2. Passage into the circulation, probably through the lymphatic tracts (rapid staining of the mediastinal glands,—see researches of Muscatello (5), Opie (6) and Menkin (7)); and subsequent elimination

by way of the biliary tract in all probability (lack of staining of the urine, red coloring of the feces, absence of coloring of the intestinal walls and of the gastric contents, red coloring of the intestinal contents). Similar findings were also obtained in rabbits stained by intravenous injections. The elimination was like that of brilliant vital red (Smith (2)), which from many points of view resembles Congo red and vital red.

3. Slight diffusion through the capillary vessels of the subcutaneous and peri-articular connective tissues and disappearance of this coloring after from 3 to 4 days.

4. No "vital" staining of the reticulo-endothelial system. However, it seems that Congo red in pathological conditions (tuberculosis, Wedekind (8) and Löwenstein (9); sepsis, Adler and Reimann (10)) is a vital dye. It is an acid dye like most other vital stains, and of the benzidinic series.

5. A long permanence *in situ*, in the case of subcutaneous injections of the dye.

EXPERIMENTAL

Material Used.—(*a*) Merck's Congo red, 1 per cent in H_2O.

(*b*) Solution of mono-urate of sodium prepared according to Rondoni (11) containing about 1 mg. of mono-urate for each cubic centimeter (titration with $KMnO_3$ N/20) at pH 7.4, as determined colorimetrically by Michaelis' method and electrometrically.

(*c*) Solution of uric acid with 1 per cent of glucose and Li_2CO_3 0.28 per cent, according to Koehler (12), containing 1 cg. of uric acid for each cubic centimeter, at pH 8–8.2.

(*d*) Controls.—Buffer solutions of phosphates according to Sörensen at corresponding pH; solutions of electrolytes at various pH; solutions of glucose and Li_2CO_3, as in the solution of Koehler; inert granular materials.

Method of Injection.—The most clear cut results were obtained by injecting at the same time both the uric acid and the dye (1 cc.) into the peritoneal cavity, killing the animals after 18–24–36 hours. Repetition of the dose was undesirable because of the inflammatory action on the peritoneum of uric acid and the consequent fixation of the dye (Menkin (7)). Studies of the speed of absorption of the dye from the subcutaneous tissue in the presence of uric acid yielded complicated results because of local inflammatory manifestations.

Results.—With intraperitoneal injections of Congo red at the same time as uric acid, an intense staining was found when the animals

were killed after 24 hours. It was diffuse in the subcutaneous tissue and especially marked in the peri-articular connective tissue. In control animals, *i.e.*, in animals injected with the control solutions instead of uric acid, only a slight rosy shade of the connective tissues could be obtained.

In the case of subcutaneous injections in corresponding situations, of Congo red plus uric acid, and of Congo red plus control material, we found a greater extension of the surface and depth of the red blotch where uric acid had been introduced, and this blotch disappeared sooner than that on the control side. It is uncertain whether this is due to a larger surface of absorption or to a higher permeability.

DISCUSSION

The interpretation of these results is not simple. Has the uric acid by itself modified the permeability? Or has it brought into play other substances influencing the capillaries? The skin is rich in histamine (Thorpe (13)) and in that histamine-like substance, if it is not true histamine, which goes under the name of the H substance of Lewis (Kalk (14)). Storm van Leeuwen (15) has shown that uric acid can exercise an activating or sensitizing action on various allergic substances, which often behave like shock-poisons. An increased concentration of the circulating dye due to modifications of its elimination must also be thought of. I did not notice any histological modifications of the liver or of the kidneys, but there may have been some functional modifications of these organs. The fact must not be overlooked that the subcutaneous and the connective peri-articular tissues showed a relatively great coloring. Are the capillary vessels of the connective tissues the most permeable? This tissue is largely influenced by modifications of the ion-concentration of the medium (Rous (16)) and may act as a "compensation-compartment" for H-ions present in abnormal quantities in the organism (Chini (17)). Recent researches of Melli (18) have shown that electrolytes introduced direct into circulation are quickly fixed in the subcutaneous tissues. Perhaps in my experiments the uric acid was in a higher concentration in the connective tissues than elsewhere. However, Bergami (19) has shown that a rapid and diffuse fixation of uric acid takes place in the kidneys of rabbits. The behavior of the peri-articular tissues is of particular interest.

It has seemed advisable to carry out some control researches *in vitro*.

Diffusion of Dyes through Gelatin as Affected by Uric Acid

Schulemann (20) noted that the speed with which some dyes are diffused in gelatin is proportional with the speed of their passage through the capillary walls, a fact afterwards confirmed also by

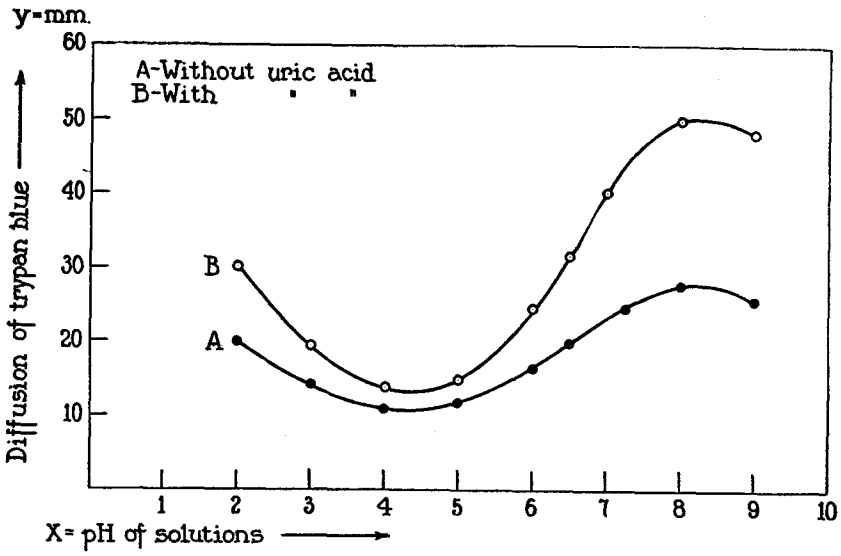

CHART 1. Diffusion of trypan blue in 5 per cent gelatin at different pH of buffer solutions, with or without the presence of uric acid (Koehler's solution). y = millimeters of column of diffusion of the dye. x = pH of buffer solutions.

Krogh (21). We must thank Höber (22) and his pupil Gellhorn (23) for systematic studies on this subject.

I have used bacteriological gelatin which had been dialyzed for 24 hours (Bechhold (24)), brought to various concentrations in H_2O, filtered when hot and distributed in equal amount in graduated bacteriological test tubes. The level attained was marked and represented the zero line. A 0.5 cc. of 1 per cent trypan blue or Congo red in H_2O was poured on the solidified gelatin, with or without uric acid (in solution or suspended). Numerous controls were prepared. The following is a scheme of the experiment.

To a series of test tubes containing 5 per cent gelatin are added 0.5 cc. of trypan blue or Congo red solution plus 2 cc. of M/3 phosphate mixture according to Sörensen at various pH (pH 4.8–5.8–6.8–7.2–8.8) plus 1 cc. of Rondoni's solution or that of Koehler. Instead of this last the controls received 1 cc. of H_2O or of physiological salt solution. Other controls were prepared with solutions of various electrolytes at various pH in order to avoid the influence of the phosphate ions on the precipitation of the uric acid (Rondoni (11)); and with granular inert materials.

The column of diffusion of the dye in the gelatin in a given time and at a constant temperature was measured from the zero line in millimeters. Also the grade of diffusion of the dye in gelatin at various dilutions was noted. Bechhold (24) advises breaking up the gelatin into various segments, liquefying it and then making comparative colorimetrical readings.

Results.—In conformity with the *in vivo* observations the uric acid was found to favor a greater diffusion of the dyes in the gelatin, not only under conditions of acidity but also under those of alkalinity. This diffusion proved to be a function of the concentration of uric acid.

Certain facts complicated the results even in the controls:

1. The speed of diffusion of the dyes varied inversely as the concentration of the gelatin; graphically, a curve is obtained of a linear relation.

2. When the concentration, time and temperature are constant, the speed of diffusion is a function of the pH of the solution. We may represent this behavior graphically by a partially parabolical curve with a minimum around pH 4–5, and with a greater development towards high values of pH (Chart 1). As the minimum of diffusion corresponds with the values of pH near those of the isoelectric point of the gelatin, it is probable that the diffusion has a relation to the state of imbibition of the colloid, which is naturally at a minimum at the isoelectric point. For the gelatin, the latter corresponds to $[H] = 2.5 \times 10^{-5}$ (Schade), then $[H] = -5 \times 10^{-0.39} = 10^{-5+0.39}$, therefore pH = 4.61.

3. In the tubes of gelatin in which alkaline mixtures of phosphates or the uric acid solution of Koehler have been stratified, phenomena of periodic precipitation can be noticed in the middle of the gelatin, which probably have an influence on the diffusion of the dye.

Some records of observations follow (Tables I and II).

The difference in the grade of diffusion, between the tubes of the gelatin, with or without uric acid solutions, is quite evident, even when the controls are varied by adjusting the pH, and introducing various electrolytes or mixtures of NaOH plus citrate of sodium, according to Sörensen.

TABLE I

Diffusion of Trypan Blue 1 Per Cent in H_2O, at Different Gelatin Concentrations

	No. of test tubes							
	I	II	III	IV	V	VI	VII	VIII
Gelatin at 10 per cent, cc.	20	18	16	14	12	10	8	6
H_2O, cc.	—	2	4	6	8	10	12	14
Concentration per cent of the gelatin.	10	9	8	7	6	5	4	3
Diffusion of trypan blue after 4 days, in mm.	4	5	6	8	10	12	17	22

TABLE II

Diffusion of Colloidal Dyes Together with Buffer Solutions with or without the Presence of Uric Acid (See Also Chart 1)

	5 per cent gelatin in test tubes					
	I	II	III	IV	V	VI
pH of buffer solutions.	3.8	4.8	5.8	6.8	7.8	8.8
Diffusion of trypan blue, mm.	15	13	15	25	28	25
Diffusion of trypan blue with uric acid (Koehler's solution).	18	15	20	45	52	50
Diffusion of Congo red.		7	9	14	17	13
Diffusion of Congo red with uric acid (Koehler's solution).		10	16	17	19	20

Dialysis Experiments

Dialysis experiments carried out through an animal membrane (fish's air bladder) have clearly shown the influence of uric acid on the diffusion of trypan blue.

Five dialysis bags, which had proved impermeable to trypan blue beforehand, were placed in a like number of flasks, each containing the same quantity of H_2O. The conditions and the results are given in the following table (Table III).

TABLE III

Diffusion through Animal Membranes of Trypan Blue Solutions with Various Admixtures

No. of dialysis	Experiment	Coloring of dialyzed fluids			Colorimetrical readings (mm.)			Observations
		After 24 hours	After 48 hours	After 96 hours	After 24 hours	After 48 hours	After 96 hours	
1	0.5 cc. of trypan blue solution + 2 cc. of Koehler's solution	++	+++	++++	8	6	2½	
2	0.5 cc. of trypan blue solution + 2 cc. physiological solution	−	−	−	−	−	−	No diffusion
3	0.5 cc. of trypan blue solution + 2 cc. 1 per cent glucose	−	−	−	−	−	−	No diffusion
4	0.5 cc. of trypan blue solution + 2 cc. (1 per cent glucose + 0.28 per cent Li_2CO_3)	+−	+−	+−	40	40	40	
5	0.5 cc. of trypan blue solution + 2 cc. 1 per cent uric acid suspension in H_2O	+−	++	++++	50	8	2	

Colorimetrical readings with Duboscq colorimeter modified by Leitz.

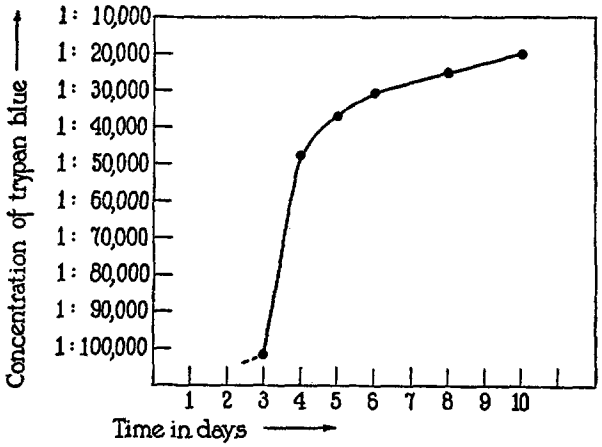

CHART 2. Concentration of trypan blue in the dialyzed liquid compared to standard solutions between concentrations 1:100,000–1:10,000. In the dialysator 0.5 cc. of 1 per cent trypan blue solution had been suspended with 2 cc. of Koehler's solution.

The colorimetrical reading could not be made with a standard sample of trypan blue, because the dialyzed fluid had taken a violet shade during the first days, which does not appear in saline or H_2O solutions of trypan blue, even when diluted to the extent of 1:1,000,000. I have noticed a certain metachromasia of the trypan blue also in the first grades of diffusion of the color in the gelatin (pomegranate red color), which was very marked in alkaline vehicles and absent in acid vehicles. After 2 or 3 days, the concentration of the trypan blue in the liquid dialyzed when uric acid is present, is such as to take the characteristic blue color, and it can therefore be compared with the standard samples of trypan blue (comparison is possible with concentrations of more than 1:100,000 (Chart 2)).

In collateral research, it has been established that the pH of the mixture (phosphates) subjected to dialysis as well as granular materials by themselves have no influence on the diffusion of trypan blue.

CONCLUSIONS AND SUMMARY

1. Congo red injected *in vivo* together with uric acid gives rise to more intense and diffuse red coloring in rats, especially in the subcutaneous and peri-articular tissues, than is the case in control rats injected simply with dye.

2. Uric acid added *in vitro* to solutions of Congo red or trypan blue increases the speed of diffusion of these dyes, both through gelatin and the animal membranes (dialyzers).

These results support a view long maintained by Professor Rondoni (25), namely, that some factor of an endothelial-capillary nature must be taken into consideration in manifestations of hyperuricemia and of gout.

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EXPLANATION OF PLATE 1

FIG. 1. *On the left*: Rat injected in the peritoneal cavity with 1 cc. of 1 per cent Congo red solution + 1 cc. of physiological solution. *On the right*: Rat of same weight injected also in the peritoneal cavity with 1 cc. of 1 per cent Congo red solution + 1 cc. of uric acid solution of Koehler. Rats killed after 18 hours from injection. Stronger staining of subcutaneous tissues in the rat having received uric acid in addition to dye.

FIG. 2. Diffusion in the 4 per cent gelatin of trypan blue solution with buffer solutions of phosphates at different pH:

1 and 1′ = pH 7.8
2 and 2′ = pH 8.8

1′ and 2′ with addition of uric acid (Koehler's solution).

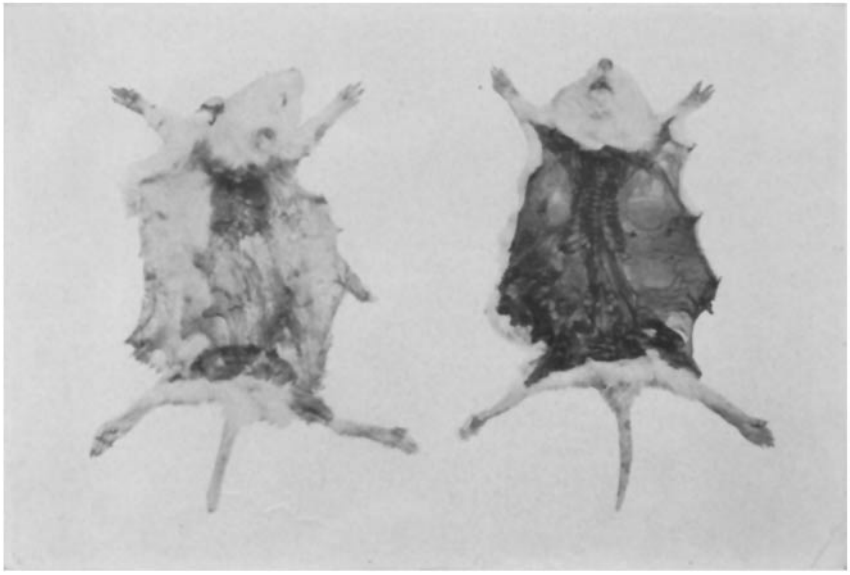

FIG. 1

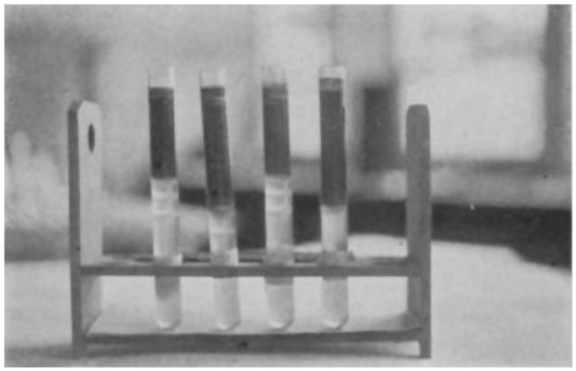

FIG. 2

(Chini: Uric acid and permeability of membranes)