Concentrations of Cl in fiber water, calculated as (Cl- minus inulin-space) (conc. Cl in plasma water)/(muscle water minus inulin-space), are matched with plasma K (chloroplatinate analysis[7]) in Table 1. The correlation is + 0.71 with a P value equal

TABLE 1

PLASMA K AND MUSCLE FIBER CL IN RATS
MM./L. OF PLASMA OR FIBER WATER

Group A		Group B	
Plasma K	Fiber Cl	Plasma K	Fiber Cl
6.9	4.9	8.8	7.7
7.0	10.7	10.7	10.3
7.4	1.9	10.8	8.2
8.5	9.0	11.8	7.0
8.1	8.0	- -	10.0
8.3	7.0	12.4	12.1
8.6	2.5	14.2	11.6
10.1	9.0	14.7	12.9
14.9	13.2	15.9	11.2

to 0.0014 that the scatter result from uncorrelated material. From Boyle and Conway, $Cl_1/d_1 = Cl/d$,[8] and if in the rat as in the frog $d_1 = K$, then $Cl_1 = (Cl/d)K$. The regression equation for the combined data of Table 1 is $Cl_1 = 0.28 + 0.795\ K$. With $(Cl/d) = .795 = 107.5/d$,[9] $d = 135.2$ and $d - Cl = 27.7$, a very likely value for diffusible anions other than Cl in plasma, particularly bicarbonate.

The negative correlation − 0.21, P = 0.20, between plasma Cl and fiber Cl/unit dry weight, though determined over a limited range of plasma Cl values, does not support adsorption of Cl as such. It may mean, if any of the plasma *K* in nephrectomy comes from endogenous source in company with anions other than *Cl*, a reduction of plasma *Cl* as this *Cl* enters the fibers with *K*. Such transfer may explain instances of fall in plasma *Cl* when the kidney is excluded and plasma *K* elevated: chronic nephritis, adrenaline release, hemorrhage, shock, asphyxia, local venous stasis, etc.; and, of course, the opposite when plasma *K* is lowered: excess cortical hormone, anesthesia.

The muscles of group A contained an inulin-space 15.8 per cent. larger than those of B. This suggests the inability of *K* compared to *Na* to support interstitial fluid bulk even after nephrectomy. Entrance into fibers of *K* as KCl from fluids containing *K* substituted for Na naturally entails entrance of water because of hypotonicity. Entrance of *K* in exchange for *Na*, assuming equal ionization inside, would involve no water shift.

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[7] W. S. Wilde, *Jour. Biol. Chem.*, 128: 309, 1939.

[8] Italicized symbols represent "activities"; d = sum of diffusible anions; subscripts indicate "fiber" ions, other ions being interstitial.

[9] 107.5 is mean plasma water Cl for all rats.

PIGMENT PRODUCTION BY TUBERCLE BACILLUS IN THE PRESENCE OF P-AMINOBENZOIC ACID

PURSUING studies on the chemotherapeutic relationship between Mycobacterium tuberculosis and fungi,[1] an intensive yellow pigmentation has been observed in cultures of a certain strain of *Tubercle bacillus* when grown in the presence of high concentrations of p-aminobenzoic acid (PABA). That the formation of this kind of pigment apparently is not restricted to the *Tubercle bacilli* and has perhaps a general significance is suggested by a recent publication by Spink and Vivino.[2] These authors described the development of a yellow pigment in cultures of staphylococci when grown in the presence of sulfa drugs.

Strain No. 607 of the American Type Culture Collection, growing first in Sauton's, later in Long's medium, was employed for the study reported here. Formation of the yellow pigment takes place in PABA concentrations of 1:1000 to 1:3000 after an incubation of about one week. At first the color is a bright yellow, but the cultures often gradually become brownish. Numerous conglomerations of yellow crystals are visible microscopically between and around the bacilli which occasionally grow like fungi in long threads and chains. No similar coloration could be observed in the presence of sulfathiazole, sulfathiourea, aniline, sulfanilic acid or paraphenylenediamine.

Benzoyl-γ-(2-methylpiperidino)-propanol hydrochloride, selected as an example of an amine-free derivative of benzoic acid, was without effect; by contrast, p-aminobenzoyl-diethylaminoethanol hydrochloride (Procaine) gave a very intense color if concentrations of 1:250 to 1:500 were used. (Notice that this quantitative relation between PABA and Procaine is about the same as in their antisulfonamide activities.)

Since the tubercle bacillus forms considerable amounts of riboflavin under identical conditions, I first considered the possibility of an increased riboflavin formation due to the presence of PABA; my recent findings, however, indicate that the yellow pigment here described is not identical with Vitamin B_2. In the absence of PABA no pigment is formed if the magnesium content of the medium is changed or if mercuric chloride is added to the medium. It is known that changing the concentration of magnesium or addition of $HgCl_2$ increases considerably the riboflavin formation by *Aspergillus niger*.[3]

The pigment is insoluble in ether, chloroform or petrol ether, but it is highly soluble in concentrated

[1] R. L. Mayer, *Revue Medical France*, December, 1941, 3–19; see C.A. 36: 5199, 1942.

[2] W. W. Spink and J. J. Vivino, SCIENCE, 98: 44, 1493.

[3] G. S. Kitavin, *Cpt. rend. acad. Sci. URSS*, 28: 517, 1940.

acetic acid and phenol. Under the microscope, tubercle bacilli containing this pigment reduce ammoniacal $AgNO_3$ and Fehling's solution.

Studies are under way in order to determine the possible relationship of this pigment to a metabolite derived from PABA and especially to factors of the vitamin B complex.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

GLYOXAL, A NON-IRRITATING ALDEHYDE SUGGESTED AS SUBSTITUTE FOR FORMALIN IN HISTOLOGICAL FIXATIONS

MANY histologists who find the traditional formaldehyde solution distressing to the eyes and nose would doubtless welcome a less offensive hardening agent of equal efficiency. Glyoxal, or oxalic aldehyde, which has recently become available[1] in fair quantities, appears very promising in this respect.

Glyoxal, whose molecule consists merely of two linked aldehydic groups, and is the simplest of the dialdehydes, has been recommended by the manufacturers as an "insolublizing agent" for protein materials such as casein, albumin, gelatin and glues. This suggested to us that perhaps the dialdehyde could also be applied for hardening animal tissues in histological fixation.

A variety of tissues of the mouse was tried in different strengths of glyoxal. Reasoning that the ordinary "10 per cent. formalin" is approximately a 4 per cent. formaldehyde solution, and that glyoxal has two aldehyde groups, one would expect a 2 per cent. solution to be about right. Liver, kidney, muscle of leg, tongue, heart, skin, spleen, lung, brain and fatty tissue of the breast of mouse were used and human breast tissue and brain. With small blocks of tissue, we found the 2 per cent. glyoxal to compare very favorably with 10 per cent. formalin, with the possible exception of muscle. Nuclei were well stained in all specimens. Even a 1 per cent. glyoxal solution was adequate for small samples of many tissues. Concentrations much higher than 2 per cent. were not suitable for small blocks. The addition of acetic acid yielded poorer results. Controls were run with 10 per cent. formalin. Only haematoxylin-eosin stain was employed, no difference being noted in the coloration of tissues between those fixed in formalin and in glyoxal.

When large masses of tissue, such as an entire human brain, were fixed, 10 per cent. concentration and slightly longer time was necessary. Either the weaker solution was exhausted or the larger glyoxal molecule does not diffuse as readily as formaldehyde.

Glyoxal is now supplied as a crude 30 per cent. to 40 per cent. aqueous solution, deep yellow and syrupy in consistency. It is quite impure and too acidic to be used without treatment. Dilute the crude solution to 10 per cent. concentration with tap water. Add powdered calcium carbonate, and stir until effervescence ceases. Frothing is severe but can be controlled by adding a little ethyl ether. Filter by suction through a rapid crepe paper. Pass through a second time if not clear. Dilute further to 2 per cent., or as desired. The final solution will be still faintly acidic (to litmus) and should be left so, never alkaline. It has only a weak odor and is not irritating.

A 30–40 per cent. crude solution of glyoxal sells for one dollar a pound in lots of a gallon or under. However, at 2 per cent. dilution it costs only about 10 to 15 cents a liter. It is expected to become cheaper and more abundant, but even now 50 gallons of the 30–40 per cent. concentration can be purchased on one order.

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[1] Carbide and Carbon Chemicals Corporation, 30 East 42nd St., New York. We are most grateful to F. J. Rauscher of the St. Louis office for generous samples of glyoxal solution.

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