or pseudo-cholinesterase predominates in various tissues or body fluids.

Recently we have found[3] that acetyl-beta-methylcholine is hydrolyzed only by true cholinesterase and not by pseudo-cholinesterase, whereas benzoylcholine is hydrolyzed only by pseudo-cholinesterase and not by true cholinesterase. These facts make it possible to estimate quantitatively the activities of the true cholinesterase and the pseudo-cholinesterase separately in any mixture of the two enzymes.

Using this method determinations were carried out on the same organs of various species in order to ascertain which type of cholinesterase is present.

Since cholinesterase plays an essential role in the chemical transmission of nerve impulses,[4] it was deemed of great importance to find out which of the two enzymes is present in brain tissue. Gray and white matter from the brain of representatives of all the vertebrate classes were investigated. In addition, various other organs, such as parotid gland and pancreas, of several mammalian species were tested. The results are tabulated below (Table 1).

TABLE 1

TYPE OF CHOLINESTERASE PRESENT IN:

	Brain	Parotid gland	Pancreas
Rat	true		
Mouse	true		
Guinea pig	true	pseudo-*	
Rabbit	true	true	
Dog	true	mixture	pseudo-*
Cat	true	mixture	true
Cow	true	true	
Pig	true	pseudo-*	
Chicken	true		
Turtle	true		
Frog	true		
Carp	true		

* Traces of true cholinesterase present.

The results listed in Table 1 show that the brains of all the vertebrates tested contain only true cholinesterase and no pseudo-cholinesterase. This fact demonstrates that in brain tissue it is the true cholinesterase which performs the function of hydrolyzing acetylcholine after it has been liberated.

The other organs tested, however, show no regularity as to the type of cholinesterase present, *e.g.*, the parotid glands of various species may contain true cholinesterase or pseudo-cholinesterase or both. Similarly, the pancreas of the cat contains only true cholinesterase, while that of the dog contains only pseudo-cholinesterase.

Summary: Brain tissue of all vertebrates contains only true cholinesterase.

No general statement concerning the type of cholinesterase in any other organ can be made, since true cholinesterase may be present in a particular organ of one species and pseudo-cholinesterase in the same organ of another.

BRUNO MENDEL
HARRY RUDNEY

BANTING AND BEST DEPARTMENT OF MEDICAL RESEARCH, UNIVERSITY OF TORONTO

[3] B. Mendel, D. B. Mundell and H. Rudney, in press (*Biochem. Jour.*)

[4] B. Mendel and R. D. Hawkins, in press, *Jour. Neurophysiol.*, 99.

ENTRANCE OF CHLORIDE WITH POTASSIUM INTO LIVE RAT MUSCLE FIBERS—CL-SPACE ERROR

THE muscle of animals after adrenalectomy with high plasma potassium contains more extracellular fluid[1,2] expressed as Cl-space (*liters* of *plasma water* calculated to contain all Cl in 1 kg muscle) than after DOCA therapy[3] when plasma K is low. This result, surprising in view of Na retention in the latter case, may be elucidated by, in fact, is a good test of, the Boyle and Conway[4] theory, in which Cl enters muscle fibers as KCl in a Donnan equilibrium. Tested to date only in excised and immersed frog muscle, the theory is now tried in live rats. Conditions predicted to increase fiber Cl were imposed: a doubled plasma K and an 11 per cent. increase in fiber water. Fiber Cl, designated as Cl- minus inulin-space, increased 75 per cent. The error involved by designation of extracellular fluid as Cl-space, error expressed as (Cl- minus inulin-space)/(inulin space), is 44 per cent. before, 58 per cent. after imposing the condition. Thus the Cl-space error is not only absolute[5] but also dynamic or functional. Thiocyanate-space should involve similar errors.

Of rats nephrectomized under ether, litter mate A received intraperitoneally 66 ml/kg of a solution containing 3.75 gm per cent. inulin or equivalent sucrose, 150 mM Na, 125 mM Cl, 25 mM bicarbonate; in mate B, 50 mM K replaced equivalent Na and only this rat received drinking water during the 22-hour equilibration. Only sheet muscles taken under amytal from the thighs were stripped into slender pieces and cleared of connective tissue, fat, and visible blood. The *same* zinc filtrate of muscle or plasma gave aliquots for the Volhard chloride and the Seliwanoff color reaction for inulin or sucrose as fructose.[6] The small uniform muscle "fructose" blank was deducted. Equilibrium was attained as revealed in comparable inulin- and sucrose-spaces and in comparable chloride and inulin values for plasma and the occasional peritoneal water.

[1] A. H. Hegnauer and E. J. Robinson, *Jour. Biol. Chem.*, 116: 769, 1936.

[2] D. C. Darrow, H. E. Harrison and M. Taffel, *Jour. Biol. Chem.*, 130: 487, 1939.

[3] D. C. Darrow and H. C. Miller, *Jour. Clin. Investigation*, 21: 601, 1942.

[4] P. J. Boyle and E. J. Conway, *Jour. Physiol.*, 100: 1, 1941.

[5] L. V. Heilbrunn and P. G. Hamilton, *Physiol. Zoöl.*, 15: 363, 1942.

[6] K. Steinitz, *Jour. Biol. Chem.*, 126: 589, 1934.

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.

WALTER S. WILDE

DEPARTMENT OF PHYSIOLOGY,
SCHOOL OF MEDICINE,
LOUISIANA STATE UNIVERSITY

[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.