is affected by a relatively small inhibition of the true cholinesterase activity. On the other hand, a small inhibition of the true cholinesterase appears to have no measurable influence on the transient depolarization produced by the intermittent release of acetylcholine at the synapses in the central nervous system, myenteric plexus and ganglia and at the myoneural junctions in the skeletal muscles. As suggested by Shelley⁴, the high acetylcholine content and the relatively low cholinesterase concentration in the longitudinal muscle of the small intestine may also be important factors in determining the high sensitivity of this tissue to inhibition of the true cholinesterase.

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- ¹ Mendel, B., and Rudney, H., *Science*, **100**, 499 (1944). Hawkins, R. D., and Gunter, J. M., *Biochem. J.*, **40**, 192 (1946). Hawkins, R. D., and Mendel, B., *Brit. J. Pharmacol.*, **2**, 173 (1947).
² Mendel, B., and Rudney, H., *Biochem. J.*, **37**, 59 (1943).
³ Grob, D., Lilienthal, jun., J. L., and Harvey, A. M., *Bull. Johns Hopkins Hosp.*, **81**, 245 (1947). Koelle, G. B., Koelle, E. S., and Friedenwald, J. S., *J. Pharmacol.*, **100**, 180 (1950).
⁴ Shelley, H., *Brit. J. Pharmacol.*, **10**, 26 (1955).
⁵ Symposium on "Anticholinesterases", *Nature*, **172**, 841 (1953).
⁶ Hawkins, R. D., and Mendel, B., *Biochem. J.*, **44**, 260 (1949).
⁷ Feldberg, W., and Lin, R. C. Y., *J. Physiol.*, **111**, 96 (1950). Feldberg, W., *J. Physiol.*, **113**, 483 (1951).

Concentration of *Brucella* Vaccine

FROZEN-DRIED *Brucella* vaccine is in various countries distributed in multiple-dose containers for economy of production and convenience in use. The number of viable, normal organisms per ampoule determines the number of subcutaneous doses one ampoule contains¹.

The dosage per ampoule may be raised by a simple, inexpensive and labour-saving technique.

Sodium-carboxy-methyl-cellulose as a substance with protective qualities which does not encourage the growth of micro-organisms had given some promise as preservative in viable liquid *Brucella* vaccine². It was also found to be an agglutinant for *Brucella* organisms. Combination of these two properties suggested its use for concentrating suspensions of organisms before freeze drying.

Carboxy-methyl-cellulose (commercial, high viscosity, type '70') was added to the *Brucella* vaccine suspension in the form of a sterile watery solution and at the rate of 16 gm. per 4,000 ml. containing approximately 6×10^{14} viable cells³. After agitation in a 5-litre flask, this was held at 4.0° C. for 18 hr. to allow for sedimentation of the cells. The supernatant was siphoned off and *mist. desicans* was added to the sediment. The mixture was pipetted into ampoules and freeze-dried on an Edwards Model No. 3 apparatus.

Pre-drying counts showed $1,375 \times 10^9$ viable organisms per ml., and a 50 per cent survival-rate was obtained when the ampoule was sealed in pure nitrogen after freeze-drying. One half ml. dried in a 1.0 c.c. ampoule thus contained 337×10^9 organisms or five doses of vaccine by the conventional standards.

Carboxy-methyl-cellulose is harmless, inert and commonly taken internally. Parenterally it produced no effect in 0.1 per cent solution under the skin. Biological reactions of the concentrated vaccine in guinea pigs and cattle have given results identical to those obtained with vaccine made from agar sur-

face culture. It was tested only in the prescribed density of 12×10^8 organisms per ml.

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¹ Joint F.A.O./W.H.O. Exp. Com. on Brucellosis: Sec. Rep., W.H.O. Tech. Rep. Ser., **67**, 13 (1953).

² Van Drimmelen, G. C., *Tydskr. vir Wetensk. en Kuns.*, **14**, 227 (1954).

³ Van Drimmelen, G. C., *J. S. Afr. Vet. Med. Assoc.*, **23**, 149 (1952).

Hæmoglobin E in Indonesia

FIVE abnormal forms of human hæmoglobin, hæmoglobin S, C, D, E and G, have been described in addition to the normal adult (A) and foetal (F) hæmoglobins.

Hæmoglobin E, which until recently was thought to be rare, has been found to have a high incidence among the Thais¹. Its pattern of incidence may be of much anthropological importance. In the present communication I am now reporting the finding of hæmoglobin E among the Indonesian peoples.

In a previous communication, cases of Cooley's anaemia in Indonesian children have been described². In order to identify the exact nature of this congenital anaemia, I made an electrophoretic study of the hæmoglobin in the erythrocytes of these individuals. At the same time a survey of pathological hæmoglobins among Indonesians was carried out.

The method employed was a slight modification of that described by Smith and Conley³ using veronal buffer pH 8.6 and ionic strength 0.05. The hæmoglobin solutions were run 8-14 hr. at room temperature.

Five hundred samples of blood taken at random from patients at the Central General Hospital in Djakarta were examined. Most of the patients were from west Java, and the rest were from different parts of Indonesia; 479 were Indonesians, 13 were of mixed Chinese and Indonesian blood, and 8 were Indo-Europeans. Eighteen individuals showed a pathological hæmoglobin in addition to normal adult hæmoglobin. This pathological hæmoglobin has exactly the same electrophoretic mobility as the hæmoglobin E we received from Thailand (hæmoglobin C is slower and hæmoglobin S migrates faster). Its visible and ultra-violet spectra were the same as those of adult hæmoglobin. I therefore diagnosed hæmoglobin E.

Of the eighteen individuals carrying hæmoglobin E in their blood, sixteen were Indonesians, one was of mixed Chinese and Indonesian blood and one was an Indo-European. The incidence among the 500 individuals examined was therefore 3.6 per cent. No other pathological hæmoglobins were found electrophoretically.

Hæmoglobin solutions prepared from the erythrocytes of cases described as Cooley's anaemia in my earlier paper² were also examined. Two of the cases have only adult and foetal hæmoglobin, which is the pattern expected in Cooley's anaemia. The third case