

presented later. The curves show clearly that ^{18}F behaves like calcium and strontium during the early time period (1-2 hours) when most scanning for bone lesions or abnormalities would be performed with this nuclide, but that pertechnetate and bromide ions do not.

Summary. ^{18}F and ^{85}Sr administered intravenously in rabbits and dogs yield the same information as ^{47}Ca , insofar as early distribution and kinetics of tissue uptake affect "bone scanning" with external gamma scintillation detectors. Uptake by bone spongiosa exceeds that by compacta. Muscle and skin contain large fractions of the total activity during the first hour, but muscle content drops rapidly. $^{99\text{m}}\text{Tc}$ as pertechnetate ion does not behave like the bone seeker, but closely parallels bromide ion, ^{82}Br , in its early distribution throughout leg tissues.

1. Corey, K. R., Kenny, P., Greenberg, E., Laughlin, J. S., *J. Nucl. Med.*, 1962, v3, 354.
2. Charkes, N. D., Sklaroff, D. M., *ibid.*, 1963, v4, 194.
3. DeNardo, G. L., Volpe, J. A., *ibid.*, 1966, v7, 219.
4. Charkes, N. D., Sklaroff, D. M., Bierly, J., *Am.*

J. Roent. Rad. Therapy & Nucl. Med., 1964, v91, 1121.

5. Blau, M., Nagler, W., Bender, M. S., *J. Nucl. Med.*, 1962, v3, 332.

6. Dworkin, H. J., Moon, N. F., LaFleur, P. D., Lessard, R. J., *ibid.*, 1965, v6, 360.

7. Harper, P. B., Beck, R., Charleston, D., Lathrop, K. A., *Nucleonics*, 1964, v22, 50.

8. McAfee, J. G., Fueger, C. F., Stern, H. S., Wagner, H. N., Jr., Migita, T., *J. Nucl. Med.*, 1964, v5, 811.

9. Neuman, W. F., Neuman, M. W., *The Chemical Dynamics of Bone Mineral*, Univ. of Chicago Press, Chicago, 1958.

10. Copp, D. H., Mensen, E. D., McPherson, G. D., *Clin. Orthoped.*, 1960, v17, 288.

11. Wallace-Durbin, P., *J. Dental Res.*, 1954, v33, 789.

12. Hodge, M. C., *Mineral Metabolism*, C. L. Comar, F. Bronner, eds., Academic Press, New York, 1964, v ii, part A, 573.

13. Carlson, C. M., Armstrong, W. D., Singer, L., *Proc. Soc. Exp. Biol. and Med.*, 1960, v104, 235.

14. Gamble, J. L., Jr., Robertson, J. S., *Am. J. Physiol.*, 1952, v171, 659.

15. MacDonald, N. S., *J. Lab. & Clin. Med.*, 1958, v52, 541.

16. Bauer, G. C. H., Wendeborg, B., *J. Bone & Joint Surg.*, 1959, v41B, 558.

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The Nature of Antibody in Swine Naturally Infected with Japanese Encephalitis Virus. (31669)

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The cyclic outbreak of Japanese encephalitis (J.E.) among man and pigs in Miyagi Prefecture was outlined previously(1). One of the main observations of that work was that the outbreak of J.E. among pigs was 3 weeks ahead of the one among people, suggesting that a serological survey of pigs through the summer season might be used for the purpose of prediction of J.E. outbreak in the human population. For that purpose, it would be most important to find out the first occurrence of infection among pigs. However, several serum specimens taken at an early phase of the epidemic season, *i.e.*, June

and July, showed high hemagglutination inhibition (HI) titers and it was difficult to decide whether these were due to antibodies elicited by infection the previous year or the current year.

The present study was undertaken in 1965 to determine if recent infection of pigs with J.E. virus could be diagnosed by the susceptibility of specific antibodies to 2-mercaptoethanol (2-ME).

Materials and methods. *Collection of swine sera:* As a rule, 50 serum specimens were collected weekly at a slaughterhouse in Miyagi Prefecture. All sera were obtained

TABLE I. Proportion of Pig Sera Containing HI Antibodies for Japanese Encephalitis Virus During Summer of 1965.

	Week										
	May- June	July	August				September				Oct. 3rd
			1st	2nd	3rd	4th	1st	2nd	3rd	4th	
No. of sera	284	218	50	50	50	50	50	50	31	31	30
No. of positive sera	30	13	1	2	23	45	49	49	31	31	30
Detection rate (%)	11	6	2	4	46	90	98	98	100	100	100
No. of sera treated with 2-ME	24	12	1	2	20	25	20	16	20	20	20
No. of 2-ME-sensitive sera	0	0	0	2	17	24	14	7	2	0	0
Positive rate (%)	0	0	0	100	85	96	70	44	10	0	0

from pigs which were killed at presumably about 6 months of age.

Treatment of sera with 2-ME: One volume of serum (usually 0.25 ml) was mixed with one volume of M/100 phosphate buffer (pH 7.4) and 2 volumes of 0.2 M 2-ME and the mixture was incubated for 2 hours at 37°C. After this treatment, residual 2-ME was removed from the mixture by dialysis.

Antibody titration by HI test: Swine sera were tested with the standard HI antigen prepared by the method of Clark and Casals from mouse brain infected with JAGAR strain(2). HI antibody titration was performed according to the method standardized in Japan National Institute of Health(3). Details of the procedure were fully described (1). After the treatment with 2-ME, when the titer was reduced to or below one-fourth of the original, the serum was judged to contain 2-ME sensitive antibody.

Results. Results obtained are summarized in Table I. Among the serum specimens obtained between May and July, 1965 43 out of 502 were positive in HI test. This is a common pattern following an epidemic year, reflecting residual antibody in those infected during the epidemic. HI titers in all these sera were insensitive to 2-ME.

In the first week of August, one of 50 was positive and this also resisted the action of 2-ME. In the second week of August, 2 positive sera were obtained and antibody titers of these were completely destroyed after 2-ME treatment, indicating that in these animals most, if not all, of the antibody was 19S by the criterion of 2-ME sensitivity(4-9). The frequency of positive sera rapidly in-

creased to 46% in the third week of August and to 90% in the fourth week, and the HI antibodies in 85-86% of randomly selected positive specimens obtained during this interval were sensitive to 2-ME.

Almost all sera collected from the first to the fourth week of September as well as in the third week of October had HI antibody against J.E. This result was concordant to that obtained in 1964(1). However, only 70% of the sera in the first week and 44% in the second week were sensitive to the action of 2-ME. In the third week, this rate decreased to 10%. By the fourth week of September and the third week of October, antibody titers of all specimens so far examined completely resisted the action of 2-ME.

Discussion. Although an outbreak of J.E. virus infection in man is predicted on the basis of serologic evidence that the infection has occurred in pigs(1), the actual onset of the epidemic in pigs may be obscured by the occurrence of sera from a small percent of pigs which contain chiefly 7S type antibodies and therefore presumably represent animals which might have had an earlier infection. However, the findings in the present study indicate that recent infection with J.E. virus can be diagnosed by the presence of 19S antibodies and are in agreement with observations by others that the detection of 2-ME sensitive antibodies is a useful means of early diagnosis of other virus infections(9,10). Consequently, the first appearance of 19S antibodies in pig sera could serve as an indicator of the onset of an outbreak in pigs and the ratio of sera which contained chiefly 19S antibody to those

which contained chiefly 7S antibody might indicate the stage of the epidemic.

These data also demonstrate that 19S macro-globulin HI antibodies are the first to appear in sera of young pigs naturally infected with J.E. virus and this, together with the finding that they are replaced by 7S antibodies, indicates that infection stimulates production of the same type of antibody molecules, and in the same order, as those observed in other mammalian species immunized with a variety of soluble or viral antigens (4-8). In addition, the susceptibility of the early antibodies to 2-ME suggests a practical method for handling sera employed in predicting an outbreak of J.E. in man.

Summary. When serum specimens of pigs obtained weekly from a slaughterhouse throughout the summer of 1965 were tested for their HI activity against J.E. virus, an abrupt change was seen from 4% positive in the second week to 46% positive in the third week. By the fourth week, 90% were positive. When the 2-ME sensitivity HI activity of these sera was tested, almost 100% of the specimens obtained through these 3 weeks contained 2-ME-sensitive antibody. However, sensitivity to 2-ME was reduced to 70% in

the first week of September, to 44% in the second week, and after the fourth week, none of the tested serum specimens was sensitive. Evidently, 2-ME-sensitive antibody appeared before the 2-ME-resistant antibody and the detection of 2-ME-sensitive antibody against J.E. virus in the early summer gave a reliable basis for prediction of the outbreak among human beings later in that year.

1. Konno, J., Endo, K., Agatsuma, H., Ishida, N., *Am. J. Epid.*, 1966, in press.
2. Clark, D. H., Casals, J., *Am. J. Trop. Med. Hyg.*, 1958, v7, 561.
3. *New Diagnostic Procedure for Japanese Encephalitis*, 2nd Ed., Japan N.I.H., Tokyo, 1962.
4. Nossal, G. J. V., Szenberg, A., Ada, G. L., Austin, C. M., *J. Exp. Med.*, 1964, v119, 485.
5. Svebag, S.-E., Mandel, B., *ibid.*, 1964, v119, 1.
6. Fazekas de St. Groth, S., Webster, R. G., *Cellular Biology of Myxovirus Infections*, J. & A. Churchill, LTD., London, 1964, 246.
7. Berlin, B. S., *Proc. Soc. Exp. Biol. and Med.*, 1963, v113, 1013.
8. Sell, S., *J. Immunol.*, 1965, v95, 300.
9. Schluederberg, A., *Nature*, 1965, v205, 1232.
10. Hatano, R., Hinuma, Y., *Proc. Soc. Exp. Biol. and Med.*, 1966, in press.

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Preliminary Observations on Reversal of Hypovolemia with Intravenous Fat Emulsion. (31670)

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A number of solutions have been used to reverse the symptoms of experimental hypovolemia (oligemia). The solutions studied have been either naturally-occurring or synthetic polymers varying in molecular size and composition. Of the naturally-occurring organic polymers the following have been

utilized as blood substitutes in hypovolemic studies: dextran(4-10), pectin(11,12), pectin derivative(13), gelatin(1,2,8), gelatin derivatives(8), and levulan, a fructosan(16). Polymeric glucose(3) and polyvinylpyrrolidone(4, 8) are examples of synthetic polymers which have been investigated for plasma-extending properties in the hypovolemic animal. In addition, whole blood(1,2), defibrinated blood (1), serum(1), plasma(7), and saline(1,4,12) have been utilized to reverse effectively the symptoms of experimental hypovolemia.

Miyagawa suggested(14) that preparations of glucose and cod liver oil, and other fats,

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