sults. Ultraviolet irradiation for short periods of time, heating to 56° C for 15 minutes, incubation at 37° C for 48 hours or addition of formalin 1:2000 destroyed the toxic effect. Sonic vibration for as long as 60 minutes left some of the toxic activity intact; so did repeated freezing and thawing at −70° C and 37° C, respectively. At 4° C the toxic agent was found to be stable for several weeks.

These results of intraperitoneal injections of influenza virus confirm earlier observations on the toxicity of influenza viruses.[10] They suggest that influenza virus may exert a toxic effect on various organs, although it is able to propagate only in the respiratory tract of the mouse. Similar results of a toxic effect have been noted recently by Evans and Rickard,[11] who produced lesions in the rabbit's cornea without demonstrable propagation of the influenza virus. It is felt that the toxicity demonstrated in these experiments may have a part in the pathogenesis of influenza in man. Since the strains of influenza virus mentioned have shown marked variation in their toxicity for mice it is conceivable that the difference in severity of influenza epidemics may depend on such factors. Although the etiologic agent of the 1918 pandemic has not been established and super-infections by various bacteria were widespread, it should be pointed out that severe hepatic damage was noted by some investigators in a large percentage of fatal cases studied during that time.[12] Toxic properties of certain rickettsial and viral agents have been described previously by Gildemeister and Haagen[13] and by Rake and Jones.[14]

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IMMUNOGENETIC CONSEQUENCES OF VASCULAR ANASTOMOSES BETWEEN BOVINE TWINS[1]

ALMOST thirty years have passed since Lillie[2] used the demonstrated union of the circulatory systems of twin bovine embryos of opposite sex to explain, on an endocrine basis, the frequent reproductive abnormalities of the female twin. Since the appearance of Lillie's paper, the freemartin, as the modified female is called, has become an important example of the effects of hormones on sex-differentiation and sexual development in mammals.[3] Consequences other than endocrinological of nature's experiment in parabiosis have, however, received little attention.

Estimates of the frequency of identical as compared with fraternal twinning indicate that the former is relatively rare in cattle.[4] Tests for inherited cellular antigens in the bloods of more than eighty pairs of bovine twins show, however, that in the majority of these pairs the twins have identical blood types. Identity of blood types between full sibs not twins is infrequent, as might be expected from the large number of different, genetically controlled antigens[5, 6] (now approximately 40) identified in the tests. If, therefore, the frequent identity of blood types in twin pairs can be explained neither as the result of monozygotic twinning nor as chance identity between fraternal twins, nor as the sum of these two factors, it is evident that some mechanism is operating to produce frequent phenotypic identity of blood types in genetically dissimilar twins. The vascular anastomosis between bovine twins, known to be a common occurrence,[2] provides an explanation.

Three additional, independent sources of evidence help to define the action of this mechanism. (1) One twin sire failed to transmit to any of his twenty progeny certain of the antigens found in his blood. In other words, the genotype of this bull as determined from his progeny appeared to lack factors responsible for some of the antigens found in his phenotype. Tests showed that cells containing these antigens could have been derived from his twin, whose genotype did contain the necessary factors. (2) In a case of superfecundation in cattle, involving twins of opposite sex and by different sires,[7] the twins had identical blood types, each possessing two antigens the genetic factors for which could not have come from his own sire or from the dam. Cells containing these

[10] G. Henle and W. Henle, SCIENCE, 100: 410–411, 1944.
[11] C. A. Evans and E. R. Rickard, *Proc. Soc. Exp. Biol. and Med.*, 58: 73–74, 1945.
[12] B. Lucke, T. Wight and E. Kime, *Arch. Int. Med.*, 24: 154–237, 1919.
[13] E. Gildemeister and E. Haagen, *Deutsch. med. Woch.*, 66: 878–880, 1940.
[14] G. Rake and H. P. Jones, *Jour. Exp. Med.*, 79: 463–486, 1944.

[1] From the Departments of Genetics (No. 346) and Veterinary Science, University of Wisconsin, in cooperation with the Bureau of Animal Industry, U. S. Department of Agriculture. This is part of a program aided by grants from the American Guernsey Cattle Club, the Holstein-Friesian Association of America, the Rockefeller Foundation and the Wisconsin Alumni Research Foundation. Appreciated contributions to various phases of the investigation have been made by Professor M. R. Irwin, C. J. Stormont and Mary W. Yeas. This study has been possible only through the generous cooperation of workers at many state experiment stations and of numerous private breeders of cattle.

[2] F. R. Lillie, SCIENCE, 43: 611–613, 1916.
[3] See "Sex and Internal Secretions," edited by Edgar Allen (Williams and Wilkins, Baltimore, 1939), for general discussions of and references to the literature on the freemartin.
[4] D. Sanders, *Zeit. für Züchtung B*, 32: 223–268, 1935.
[5] L. C. Ferguson, *Jour. Immunol.*, 40: 213–242, 1940.
[6] L. C. Ferguson, C. Stormont and M. R. Irwin, *Jour. Immunol.*, 44: 147–164, 1942.
[7] A description and discussion of this case will be published elsewhere by Mr. B. H. Roche, who called it to our attention and provided us with blood samples from the animals involved.

critical antigens could in each case have been derived from the co-twin. (3) It has been demonstrated, by a simple immunological technique developed for this purpose, that there is a mixture of two distinct types of erythrocytes in certain twins.

These facts are consistent with the conclusion that an interchange of cells between bovine twin embryos occurs as a result of vascular anastomoses. Since many of the twins in this study were adults when they were tested, and since the interchange of formed erythrocytes alone between embryos could be expected to result in only a transient modification of the variety of circulating cells, it is further indicated that the critical interchange is of embryonal cells ancestral to the erythrocytes of the adult animal.[8] These cells are apparently capable of becoming established in the hemapoietic tissues of their co-twin hosts and continuing to provide a source of blood cells distinct from those of the host, presumably throughout his life.

Several interesting problems in the fields of genetics, immunology and development are suggested by these observations. Most of them are still largely speculative and will not be considered here. An application that may be mentioned is the tool now provided by the blood tests for selecting, with a high degree of reliability, those heifers, born twin with bulls, that are potentially not freemartins but normal, fertile individuals. A heifer whose blood type is the same as her twin brother's will very probably be a freemartin, while a difference in even a single antigen between twins of opposite sex may indicate that vascular anastomosis did not occur, and therefore that the heifer will be normal. Thus clinical observations on the heifer alone, probably not always reliable when the heifer is young, can be supplemented by an objective laboratory test applicable as soon as the twins are born. Possible limitations of this application, as well as a more complete presentation of the data and further discussion of the implications of the present study, will be included in another paper.

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GROWTH INHIBITION BY ANALOGUES OF PANTOTHENIC ACID. II. α- AND β-SUBSTITUTED PANTOTHENIC ACIDS AND RELATED COMPOUNDS[1]

VARIOUS analogues of pantothenic acid which contain the pantoyl (α,γ-dihydroxy-β,β-dimethylbutyryl) group inhibit growth of organisms which do not synthesize pantothenic acid.[2-5] Such inhibition is competitive in nature.

Since isoserine and β-aminobutyric acid have been reported to prevent the growth-promoting effect of β-alanine for yeast,[6] the pantoyl derivatives of these compounds were prepared to determine if they would inhibit growth of bacteria by interfering in a similar fashion with utilization of pantothenic acid. A related compound, N-pantoyl-β-aminoisobutyric acid[7] has been included for comparison and testing on additional organisms. A homologue of pantothenyl alcohol,[2] N-pantoyl-4-amino-2-butanol, was also prepared and tested.

All these compounds have an asymmetric carbon atom in both the lactone and non-lactone portion of the molecule; consequently the products synthesized contained a mixture of two to four stereoisomers. To avoid separation of diastereoisomers, crude reaction mixtures were tested throughout. In all cases, adequate controls showed that the parent lactone and substituted amine from which the analogues were prepared did not show the physiological properties of the condensates.

All the above compounds inhibited the growth of microorganisms which require pantothenic acid. This inhibition was competitive in nature, since it became apparent only when the ratio of the concentration of the analogue to that of pantothenic acid surpassed a definite value, but was independent of the absolute amount of the analogue present. Two of the compounds seemed to show a slight growth-promoting activity in the absence of pantothenic acid, but inhibited growth induced by added pantothenic acid to the level of their own activity.

Details of these findings are presented below.

EXPERIMENTAL

dl-Pantothenyl Alcohol. This product was prepared as previously described.[2]

N-Pantoyl-4-amino-2-butanol. Hydrogenation of aldoxime (40 gm in 150 cc of ethanol) proceeded readily at a pressure of 2,000 lbs./sq. in. and 120° in the presence of Raney's nickel catalyst. After filtering to remove the catalyst and distilling the solvent, the residue was fractionally distilled under reduced pressure to obtain dl-4-amino-2-butanol (yield, 25 gm). The aminobutanol was condensed with dl-pantolactone in the manner described for the preparation of pantothenyl alcohol[2] from 3-amino-1-propanol. The product thus prepared contained 88 per cent. of the various stereoisomeric forms of N-pan-

[8] *Cf.* H. E. Jordan, *Physiol. Rev.*, 22: 375–384, 1942.

[1] For paper I of this series, see Snell and Shive (footnote 2).

[2] E. E. Snell and W. Shive, *Jour. Biol. Chem.*, 158: 551, 1945.

[3] E. E. Snell, *ibid.*, 139: 975 and 141: 121, 1941.

[4] J. W. Barnett and F. A. Robinson, *Biochem. Jour.*, 36: 357, 364, 1942.

[5] R. Kuhn, T. Wieland and E. F. Moeller, *Ber. dtsch. chem. Ges.*, 74: 1605, 1941.

[6] N. Nielson and G. Johansen, *Naturwissenschaften*, 31: 235, 1943.

[7] M. A. Pollack, *Jour. Am. Chem. Soc.*, 65: 1335, 1943.