of the others may be merely mentioned, for example: (a) the activity of acidified broth containing nisin was destroyed on attempting to concentrate *in vacuo*, some activity reappearing on keeping; (b) when the organism was allowed to grow at 22° instead of 30° a concentrated product active against *Strep. cremoris* but not against *Strep. agalactiae* was obtained; (c) significant yields were obtained in some media after steaming, but not after autoclaving, although the organisms still grew well; and (d) the sensitivity of nisin to certain chemicals and solvents contrasts strangely with its stability to others and to acids and heat.

It was impossible to prove that the needles observed were true crystals. Their shape in cross-section could not be observed. The slow filtering and the slightly syrupy nature of the ethanolic solutions, as well as the formation of gels when such solutions were rapidly cooled, suggests that the needles may well have been tactoids.

Although a few preliminary experiments have shown that nisin is closely associated with a polypeptide, the solubility measurements indicate that further purification is required before the properties of the active substance, or substances, can be properly investigated. Meanwhile, the purification so far achieved has facilitated experiments in other directions.

SUMMARY

1. Experiments in concentrating and purifying the antibiotic nisin are described.

2. Nisin may be obtained in the form of needles by cooling a solution in 80 % ethanol at 'pH' 4.2 ('pH' is glass electrode reading of ethanolic solution diluted with 11 volumes distilled water).

3. The properties of the 'crystalline' product have been investigated. It will inhibit the growth of the more sensitive organisms at dilutions from 10^{-8} (*Strep. agalactiae*) to 10^{-9} (*Strep. cremoris*); it is stable for weeks in acid solution and for many hours in blood at 37°; it contains the amino-acids alanine, valine, leucine, isoleucine and others, and appears to consist of a mixture of at least two polypeptides.

Special thanks are due to Benger's Ltd. (of British Chemicals and Biologicals Ltd.) for their co-operation in making, and generosity in supplying, active concentrates. The helpful encouragement given by the Director, Prof. H. D. Kay, and the invaluable support of the Assistant-Director, Dr A. T. R. Mattick, are gratefully acknowledged. Thanks are also expressed to Dr A. Hirsch for useful suggestions. The work described under 'Preparation of crude concentrates' was carried out in the laboratories of Benger's Ltd., whose Director, Mr B. D. Thornley, is to be thanked, as are also Dr E. G. Stopher, Mr C. Epstein, and Miss E. M. Barnes, members of the staff, for their assistance. The later help of Mrs C. Waterhouse is acknowledged with thanks.

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The Cholinesterase Activity of the Serum of Newborn Animals, and of Colostrum

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(Received 23 May 1949)

The activities of the enzymes in serum which split acetylcholine have been investigated fairly extensively in man, both in health and disease. Enough work has also been carried out on animal sera to establish the general level of enzyme activities in the various species, and active preparations of the so-called 'pseudo' enzyme (Mendel, Mundell

& Rudney, 1943) have been made from various tissues. It is, however, believed that no tests have hitherto been made on newborn animals, or on the colostrum and milk of their mothers. Human beings, rats, rabbits, guinea pigs, cats and dogs have been investigated in the work to be described.

METHODS

The adult humans were healthy males. Blood was taken from them by vein puncture with a minimum of stasis. The infants were of both sexes and were all full term and normal. Their mothers were also normal and had given birth to the children without assistance or anaesthetic. Blood was taken from infants at birth by puncturing the umbilical cord with a large-bore needle or by allowing the maternal end of the cord to drain into a centrifuge tube, and later from the heel by cutting out a disk of skin with a scleral trephine and allowing the blood to flow without pressure into a small tube. Blood was obtained without anaesthesia from adult animals by vein puncture or decapitation, and from young animals by heart puncture from the following species: rats (Lister Institute strain); guinea pigs and rabbits (Department of Pathology stock); cats and dogs of various breeds. At birth and during the first 7 days thereafter, blood had to be withdrawn from several rats to get enough serum for each determination. After the samples were taken, the serum was separated by centrifuging as soon as possible, with care to minimize evaporation. Puncturing the heart did not appear to harm the young animals, and, after a few days, second and even third samples of blood were sometimes taken from the same animal by this method. Colostrum and milk were obtained by manual expression.

The measurements of enzyme activity were made manometrically at 37° (McArdle, 1940), using 1 ml. of 2.5% acetylcholine chloride as substrate in a total volume of 3 ml. of fluid. The liberation of 1 μ l. of CO₂/min./ml. serum, colostrum or milk has been defined as the unit activity.

RESULTS

Man. The average activity of 28 adult sera was 76 units (s.d. 13.8), which agreed very well with the figure obtained by McArdle (1940). The mean activity of 39 cord sera was 50 units (s.d. 10.7). This difference was significant ($t = 12.50$; $P < 0.01$). The changes in activity following birth are shown in Table 1.

Table 1. *The cholinesterase activity of the sera of newborn infants*

Age	No. of samples	Average activity (μ l. CO ₂ /ml./min.)	Standard deviation
At birth	39	50	10.7
0-24 hr.	39	62	14.1
5-10 days	71	64	15.7
11-21 days	19	75	14.3
Over 3 weeks	4	87	2.7

The apparent rise in the first 24 hr. was checked on numerous occasions by taking both specimens of blood from the same baby. The increase in the average activity of the sera of the 39 babies from 50 to 62 units was statistically valid ($t = 6.93$; $P < 0.01$). This rise, however, may not represent a real change in enzyme activity, since the two specimens of blood were not taken from the same site, and peripheral blood has been shown to be more

concentrated, or at any rate to carry more haemoglobin/ml., than cord or sinus blood (DeMarsh, Alt, Windle & Hillis, 1941; DeMarsh, Alt & Windle, 1948). These observations have been confirmed. The subsequent rise of enzyme activity was to be expected at some period of early childhood, since McArdle (1940) found older children to have higher serum activities than adults and this has been confirmed by one of the authors (Hutchinson, 1949). No

Fig. 1. Cholinesterase activities in the sera of young animals.

relationship has been found between the level of activity in cord sera and the weight of the child at birth, or the length of its gestation period as reckoned from the beginning of the mother's last menstrual period. No correlation has been found, moreover, between the enzyme activity of cord sera and the mother's age or her clinical status. The level of enzyme activity of colostrum, and milk up to the 22nd day, has been found to be negligible. Many specimens had no measurable activity and only one specimen out of 30 had an activity as high as 10 units.

Rats, rabbits, cats and guinea pigs. Adult and newborn animals of these species have been investigated and the variations in the first 3 weeks of life are shown in Fig. 1. Several litters were used for the young of each species, and the points represent the mean of up to 8 specimens.

The following values had been found for the adult animals: rats, 13.1 units (mean of 6 males); rabbits, 12.2 and 11.5 units (2 males); cat, 24.0 units (1 female); guinea pigs, 40.0 and 57.0 units (2 females). The levels at birth were lower than those of the

adults in the rat and rabbit, and higher in the cat and guinea pig. Except in the guinea pig the general trend after birth was similar to that in man, in that there was a definite rise. The trend with the guinea pig was less certain.

The colostrum of one cat was investigated and found to contain less than 1 unit/ml.

Dogs. The sera of 6 normal adults, 4 of whom were males, and 2 non-pregnant females, averaged 38 units; and experiments have been made on 5 litters of puppies. The sera of puppies at birth had an activity of the same order of magnitude as the adults, but there was a rise in the first few days of life to about 800 units, and a fall in the next 2 weeks to about 50 units. Colostrum obtained from a bitch at the time of parturition was found to have an

freely from birth and gained weight rapidly. Furthermore, puppies reared on evaporated cow's milk, which contained no measurable cholinesterase activity, showed no increase in serum activity although they grew as well (and their eyes opened at the same time) as litter mates kept with the mother. This is illustrated in Fig. 2.

The activity of the bitches' serum did not rise very much post-partum although there may have been a small increase in some instances. In one animal, the serum, before delivery, had an activity of 47 units. This had risen to 74 units on the following day and remained above 60 units for at least 17 days.

The enzyme present in bitches' milk hydrolysed benzoylcholine and acetylcholine but not acetyl- β -methylcholine. It therefore corresponds to the 'pseudo-cholinesterase' of Mendel *et al.* (1943).

DISCUSSION

Until more is known of the source and function of the unspecific cholinesterase found in serum it is difficult to assess the importance of these results. It must remain for a time, therefore, an observation of fact that the activity of the sera of infants at birth tends to be below that of adults and to rise soon afterwards to levels comparable with those found in later childhood. A rise after birth seems to take place also in the rat, rabbit and cat, but the level at birth may be below or above that of the adults. Newborn guinea pigs had a higher level of activity in their sera than was found in the mature animals investigated and it did not rise after birth, and this may be connected with the advanced stage of development of this species at birth.

The dog must be considered separately for it shows a remarkable species difference in biochemical behaviour in that the colostrum contains a relatively enormous amount of cholinesterase, and it would be interesting to know at what stage of evolution this difference arose and whether it is exhibited by any allied genera and species. The evidence indicates that the source of the high activity in the puppies' sera was the colostrum of the bitch, and it seems, therefore, that the enzyme must have escaped digestion and passed through the wall of the intestine unchanged. The passage of proteins and immune bodies through the wall of the gut has been demonstrated in the young of some animals and suspected in others (Schloss & Anderson, 1922; Anderson & Schloss, 1923; du Bois, Schloss & Anderson, 1925), but, so far as we know, this is the first evidence for the passage of an enzyme. Cholinesterase is not thought to have any immunological significance nor is it known whether it plays any functional role in the mammary gland of the bitch or in the serum of a newborn puppy. Its lipolytic activity in the gut may be of value in the newborn

Fig. 2. The cholinesterase activities of puppy sera and of bitches' milk during the first 16 days after birth. A, activity of mother's colostrum and milk; B, activity in the sera of puppies feeding from the mother; C, activity in the sera of puppies fed on evaporated cow's milk.

activity of 6000 units, and this activity declined rapidly at first and then more slowly. The milk of one animal had an activity of 54 units, and of another 400 units, by the 5th day, and was still 20–30 units after a fortnight. Composite curves of the findings plotted on a logarithmic scale are shown in Fig. 2.

The colostrum or milk expressible from glands which were not being suckled remained high after the activity in the milk from other glands had fallen to a lower level. Thus the activity of a pooled sample of fluid from unused glands on the 5th day was 918 units and on the 15th day was 2000 units.

The rise in the serum activity of the puppies depended upon the consumption of the mother's colostrum, for some puppies, which did not gain weight and thrive, presumably because they had not taken sufficient milk, had much smaller increases of activity in their sera than those which took milk

puppy but would not appear to be essential since the three puppies reared on evaporated cow's milk thrive satisfactorily without it. Apart from this, however, bitches' colostrum should provide a valuable source of unspecific cholinesterase in high concentration, and it is hoped to investigate some of the problems arising out of the observations here presented.

SUMMARY

1. In newborn humans, rats, rabbits and cats the cholinesterase activity of the serum tended to be near the adult level and to rise soon afterwards to

about twice the initial value. In guinea pigs the activity did not rise.

2. The colostrum of man and of the cat had negligible cholinesterase activity.

3. In puppies the serum cholinesterase rose to 25 times its birth level in 3 days. This increase appears to be due to the very high cholinesterase activity of bitches' colostrum.

The human material was obtained from the Landes-frauenklinik, Wuppertal, through the courtesy of the Director, Prof. Anselmino. A pure-bred setter was obtained from Mr Parry, the Canine Research Station, Kennet. Our thanks are also expressed to Miss M. F. Harrison for considerable assistance with work carried out at night.

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The Glucuronide-synthesizing System in the Mouse and its Relationship to β -Glucuronidase

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(Received 25 May 1949)

The β -glucuronidase activity of mouse liver, kidney or uterus has been shown to reflect the state of proliferation of the tissue (Levy, Kerr & Campbell, 1948; Kerr, Campbell & Levy, 1949a). It would appear that β -glucuronidase is distinct from the enzyme system responsible for the production of conjugated glucuronides in the body. The work of Lipschitz & Bueding (1939) suggests that this process is more complex than a simple condensation of the aglycone with free glucuronic acid, and that it takes place in liver and kidney only. β -Glucuronidase, on the other hand, is present to a greater or less extent in practically all animal tissues that have so far been examined (see, for example, Oshima, 1934). Karunairatnam & Levy (1949) found that glucuronide synthesis by adult mouse-liver slices was not appreciably impaired by saccharic acid in con-

centrations which caused almost complete inhibition of β -glucuronidase.

The ability of various mouse tissues to synthesize glucuronides has been studied and compared with their glucuronidase activity under conditions leading to changes in the latter.

EXPERIMENTAL

Measurement of glucuronide synthesis. The conversion of o-aminophenol to its glucuronide in sulphate-free bicarbonate Ringer solution was followed by the method of Levy & Storey (1949). Except in the case of lung, the tissue was sliced, and an amount corresponding to not less than 10 mg. dry weight was taken for each estimation. If necessary, slices from more than one animal were pooled. Whenever possible, the estimation was done in quadruplicate in order to reduce the variable error in the procedure (see Levy & Storey, 1949). In the case of lung, the intact lobes were used. Boyland & McDonald (1948) have shown that this is permissible for measurements of metabolism in lung from young adult mice.

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