These Figures show that the activity of leucine aminopeptidase in the kidney is very small during the foetal period of life. Further, they show that the occurence of leucine aminopeptidase happens regionally so that some few single, widely distributed tubular groups first show a moderate enzymatic activity, while the other parts of the cortex and the medulla still are fully negative. A high increase in the enzymatic activity occurs after delivery.

The observations made are interesting from the point of view of the foetal function of the kidney. Numerous investigations have shown that the function of the foetal kidney begins at a very early stage of pregnancy. One of the functions of the kidney during foetal life is supposed to be to participate in producing foetal fluid[3]. Our figures could be interpreted to show that in a rather late stage of pregnancy only very few single nefrons are capable of functioning where the presence of leucine aminopeptidase is needed and are thus competent for full function from this point of view. The tubuli of these nefrons are also shown to contain the enzyme in very low concentration, which may indicate that the function of these nefrons also cannot be intense.

The activity of leucine aminopeptidase increased soon after delivery, which is in good agreement with the earlier observations that the functional capacity of the kidney increases very intensely during the first days after delivery.

Fig. 3

Zusammenfassung. Histochemisch demonstrierbare Leucinaminopeptidase erschien in den letzten Tagen der Schwangerschaft in einzelnen Gruppen von Tubuli der Rinde der fötalen Rattenniere. Der grösste Teil der Rinde und das Mark erwiesen sich als negativ. Nach der Geburt verbreitete sich das Ferment über die ganze Rinde und vermehrte seine Aktivität stark.

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Department of Anatomy, University of Turku (Finland), January 24, 1961.

[3] R. E. SHAW and H. J. MARRIOT, J. Obstetr. Gynaec. *56*, 1004 (1949).

Increased Serum Diphenylamine Reaction in Patients with Leukemia

Normal human serum contains a variety of carbohydrate-reacting material some of which is tightly bound to peptide and protein to form the so-called mucosaccharide fraction. This mucosaccharide fraction can be estimated by a number of colorimetric reactions based on the sugar component; a comprehensive list of these reactions is given by BYWATERS and GLYNN[1]. In particular, the diphenylamine reaction[2] has often been used for obtaining an overall value of the level of mucosaccharides in serum[3-6].

Previous work on the diphenylamine reaction of human serum has shown that there is a considerable increase in the reacting component in the serum of patients with various types of malignant disease, acute rheumatoid arthritis and tuberculosis. Although NIAZI and STATE[4] included a considerable variety of malignant conditions in their study they did not differentiate between the conditions in listing the results; all their malignancy values were grouped together under one heading. Their investigation included one leukemic patient. We thought it worth while to study leukemia in more detail in this connection when serum from leukemic patients was made available to us by the courtesy of Dr. PRANKERD and of the Radiotherapy Department, U.C.H. Since this work was started a value for the *N*-acetyl neuraminic acid content of leukemic serum has been published[7] although as yet no details of the method used etc. are available to us.

Procedure. 5 ml of blood were mixed with 0.2 ml of 5% versene and then centrifuged to remove all cellular elements. 0.5 ml of the resulting serum[8] were heated for 15 min at 90° with 9.5 ml of 5% trichloroacetic acid. After cooling the mixture was filtered and the extract was used for estimating the mucosaccharide component as follows:

2 ml extract were boiled for 1 h with 4 ml of diphenylamine reagent[2] (1 g diphenylamine in 98 ml glacial pure acetic acid and 2 ml pure concentrated sulphuric acid). After cooling the absorption spectrum was determined using a Unicam-SP-500 spectrophotometer; the region covered was 360–700 mμ and the tubes were always read against a heated trichloroacetic acid blank boiled for the same period of time.

In agreement with earlier work[4], it was found that the cold TCA-soluble fraction of serum gave little absorption at 530 mμ on boiling with diphenylamine reagent. This indicates that the majority of the material producing the peak at 530 mμ is bound to protein and is extracted by hot TCA. Figure 1 shows the absorption spectra obtained by boiling hot TCA extracts of red blood cells, leucocytes and serum with diphenylamine reagent for 1 h. It can be seen that only the serum extract shows a marked 530 mμ peak.

[1] E. G. L. BYWATERS and L. E. GLYNN, in *Biochemical Disorders in Human Disease* (Ed. R. H. S. THOMPSON and E. J. KING, J. & A. Churchill Ltd., 1957), p. 634.

[2] Z. DISCHE, in *The Nucleic Acids* (Ed. E. CHARGAFF and J. N. DAVIDSON, Academic Press Inc., New York 1955), Vol. 1.

[3] N. W. PIRIE, Brit. J. exp. Path. *17*, 269 (1936).

[4] S. NIAZI and D. STATE, Cancer Res. *8*, 653 (1948).

[5] W. AYALA, L. V. MOORE, and E. L. HESS, J. clin. Invest. *30*, 781 (1951).

[6] A. F. COBURN, L. V. MOORE, and J. HANINGER, Arch. int. Med. *92*, 185 (1953). – G. R. FEARNLEY, J. PIRKIS, N. DE COEK, R. LACKNER, and R. I. MEANOCK, Ann. Rheum. Dis. *14*, 226 (1955). – E. CECCHI and F. FERRARIS, Ann. Rheum. Dis. *14*, 267 (1955).

[7] P. BÖHM (1958) quoted by A. Gottschalk, in *The Chemistry and Biology of the Sialic Acids* (Cambridge University Press 1960), p. 93.

[8] In accordance with common practice this fraction is referred to as serum although the blood had been prevented from clotting. The sialic acid content of fibrin (and haemoglobin) is negligible compared to the serum level[7].

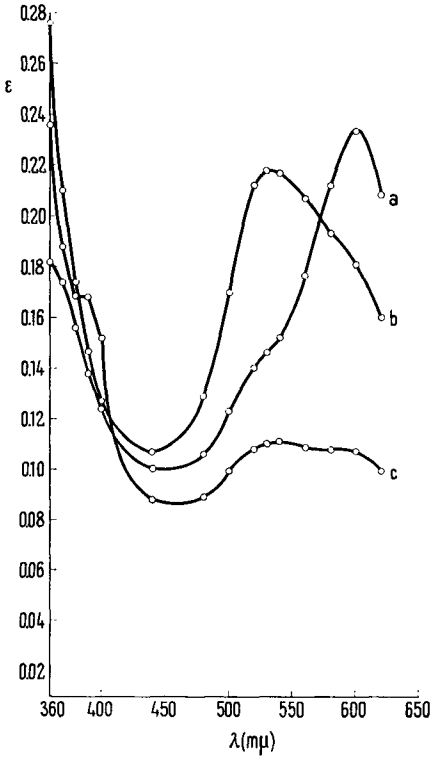

Fig. 1. Absorption spectra obtained after boiling hot trichloroacetic acid extracts of blood fractions with diphenylamine reagent for 1 h. Curve a, using an extract of leucocytes; curve b, plasma; curve c, red cells.

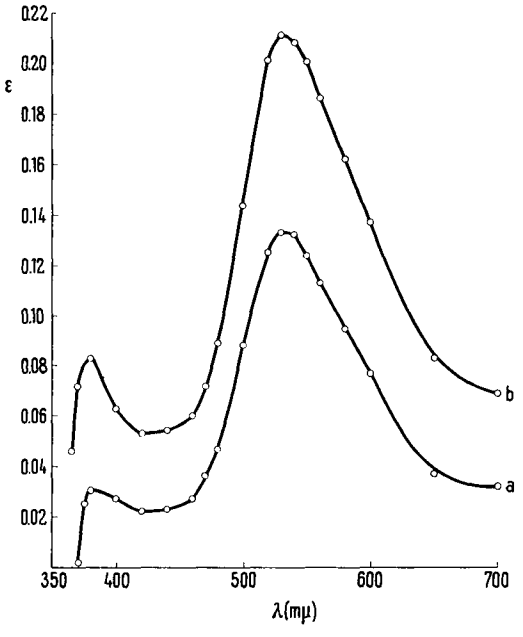

Fig. 2. Absorption spectra obtained by boiling hot trichloroacetic acid extracts of normal (a) and leukemic serum (b) with diphenylamine reagent under the conditions described in the text.

Figure 2 shows the spectrum obtained with a leukemic sample (case No. 2) and with a normal serum sample. It can be seen that there is no qualitative difference between the two spectra, both showing a marked peak at 530 mμ and a rise in absorption below 400 mμ. The 360–400 mμ region proved one of great variability, some samples showing a small peak at about 380 mμ as in Figure 2, and others a rapidly rising absorption as in Figure 1. This variation probably results from a number of factors: free glucose produces considerable absorption in this region, and sialic acid yields a secondary peak at 380 mμ.

The Table gives the readings obtained at 530 mμ with leukemic and normal sera. We have included in the Table a case of myeloid metaplasia and two cases of Hodgkins disease, one in the acute phase and the other quiescent. It can be seen that the mean value for the leukemic samples is considerably higher than that obtained with normal samples. The difference between the two mean values is statistically significant. The magnitude of the diphenylamine reaction in the two Hodgkins diseases paralleled the gravity of the condition; in the single case of myeloid metaplasia the value obtained was within the normal serum limits. Our results are therefore in agreement with the previously mentioned work of BÖHM[7] on the rise in the sialic acid content of serum in leukemia, and the generalised malignancy findings of NIAZI and STATE[4].

It is of interest to bear in mind that in most instances where an elevation of the diphenylamine reaction occurs there is also an increase in the erythrocyte sedimentation rate. Recent work[9] on the cell wall composition and

Diphenylamine reaction in normal and leukemic serum. Values are given in terms of *N*-acetyl-neuraminic acid as mg/100 ml serum. The conditions, both for the serum samples and for the calibration curve with *N*-acetyl-neuraminic acid, were as described in the text.

Case	Age	Diagnosis	White cell count ($\times 10^{-3}$)	mg/100 ml serum
1. Mrs. C.	62	chronic myeloid	240	78
2. Mr. E.	36	acute monocytic	116	96
3. Mr. S.	68	chronic lymphatic	72	93
4. Mr. A.	65	chronic myeloid	44	115
5. Mrs. W.	63	acute myeloid	32	128
6. Mr. R.	67	chronic lymphatic	109	106
7. Mr. W.	23	acute lymphoblastic	3.5	198
8. Mr. S.	58	chronic myeloid	12	72
9. Mr. F.	27	acute Hodgkins	6.8	190
10. Mr. C.	35	quiescent Hodgkins	6	107
11. Mrs. S.	68	myeloid metaplasia	3.5	61
		mean value[a] of cases 1– 8		111 ± 14
12.–19. Normals		mean value[a] of cases 12–19		61 ± 2

[a] ± standard error of the mean

[9] G. M. W. COOK, D. H. HEARD, and G. V. F. SEAMAN, Nature (Lond.) *188*, 1011 (1960).

agglutination reactions of red cells have stressed the role of sialic acid. An increase in the serum level of similar substances might therefore alter the sedimentation rate not only by affecting the physical properties of the serum itself but by actual combination with like structures on the erythrocyte membrane.

Riassunto. Gli autori riscontrano nel siero di pazienti leucemici un aumento della reazione con difenilamina rispetto al siero di soggetti normali. Questo aumento è discusso alla luce di precedenti risultati che collegano questa reazione con i mucosaccaridi del siero.

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[10] We thank the British Empire Cancer Campaign for an award to one of us (D.L.), and are grateful to Professor C. RIMINGTON for helpful comments on the manuscript. The *N*-acetyl-neuraminic acid used for the calibration curve was very kindly supplied by Miss CARROLL of The Medical Research Council, Mill Hill.

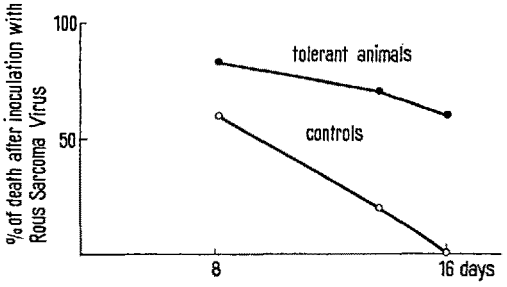
Immunological Tolerance to Rous Sarcoma Virus in Ducks

We have succeeded[1] in eliciting immunological tolerance to Rous sarcoma virus in ducks, just as it was produced in turkeys[2,3]. Intraembryonic or better repeated post-embryonic injections of chicken blood or lyophilized blood were used for the induction of tolerance. A comparison of susceptibility to Rous virus in control and tolerant ducks of different ages is shown in the Figure. The distribution of both curves suggests that although the differences in susceptibility of the two groups are apparent on the 8th day, 16 day-old control birds are resistant, whereas tolerant ducks succumb in more than 50% to inoculation with cell-free filtrates from Rous sarcoma. (Extracts were prepared by homogenization of 20% suspension of chicken Rous sarcoma tissue (stored in frozen state) in isotonic potassium citrate at pH 7, by centrifugation for 20 min at 8000 *g* and by filtration of supernatant through procelain candles 'Selas 02'.) The state of tolerance depends on repeated administration of antigen during the adaptive period. A single injection of 0.3 ml of chicken blood on the first day after hatching is capable of eliciting a weak degree of tolerance (10%), whereas the same dose divided into 3 injections administered on the first, third, and fifth day induces a clear-cut tolerance (30%). The same dependance was found to exist with the dose of 0.9 ml. The postnatal period during which it is possible to induce tolerance in ducks is relatively long—injections of chicken blood commenced on the 14th day after hatching are still fully effective whereas those commenced later are ineffective[4]. Nuclear nucleoproteid appeared to be a highly effective chicken antigenic material for induction of tolerance to Rous virus, whereas the desoxyribonucleic acid itself was ineffective[5].

The original explanation of tolerance to Rous virus, indicating that this is the tolerance displayed directly to the Rous virus particle, which therefore contains normal chicken antigens, is in conflict with more recent information concerning the nature of Rous virus. This is firstly, because it has been found that the Rous virus does not contain normal chicken antigens as an integral part of its functional surface[6], that it penetrates into the cell[7] much earlier than actively acquired immunity could play a role and that the presence itself of virus-neutralizing antibodies does not profoundly affect the tumour growth[8].

Secondly, our results given in the Table also show that virus-neutralizing capacity of sera from tolerant animals bearing large tumours does not differ from sera of resistant control animals, although in case of direct tolerance to the virus particle it must have been lower. Similar results obtained PRINCE[3] in tolerant turkeys.

All the results obtained with tolerance to Rous virus in ducks, as well as those obtained in turkeys[3,9] are in agree-



● ○ each point represents percentage of death in a group of 8 to 10 animals.

Natural susceptibility and actively acquired tolerance to Rous sarcoma Virus in ducklings of different ages.

Tests for virus-neutralizing capacity of sera from tolerant and control ducklings (tests were made on 10 day-old Leghorn chicks)

ID 50% after incubation of sera from tolerant animals with Rous virus	ID 50% after incubation of sera from control animals with Rous virus
$10^{-2,66}$	$10^{-2,5}$
$10^{-2,5}$	$10^{-2,33}$
$10^{-2,5}$	$10^{-2,16}$
$10^{-2,7}$	$10^{-2,54}$
$10^{-2,33}$	$10^{-3,0}$
$10^{-3,16}$	$10^{-3,33}$

[1] J. SVOBODA, Folia biol. (Praha) *4*, 205 (1958).
[2] M. SIMONSEN, Nature *175*, 763 (1955). – R. J. C. HARRIS, Proc. Roy. Soc., B, *146*, 59 (1956). – J. SVOBODA and M. HAŠEK, Folia biol. (Praha) *2*, 256 (1956). – R. J. C. HARRIS, J. chron. Dis. *8*, 58 (1958). – R. J. C. HARRIS and P. J. SIMONS, Nature *181*, 1485 (1958).
[3] A. M. PRINCE, Personal communication (1960).
[4] J. SVOBODA, Folia biol. (Praha) *6*, 21 (1960).
[5] J. SVOBODA, Folia biol. (Praha) *6*, 96 (1960).
[6] H. RUBIN, Virology *2*, 545 (1956). – T. BORSOS, J. Nat. Cancer Inst. *20*, 1215 (1958). – M. RYCHLÍKOVÁ and J. SVOBODA, Virology *10*, 545 (1960).
[7] H. TEMIN and H. RUBIN, Virology *6*, 669 (1958).
[8] P. VIGIER, Bull. du Cancer *45*, 460 (1958).
[9] J. HORT, J. SVOBODA, and M. HAŠEK, Folia biol. (Praha) *6*, 371 (1960).