to be tortuous. Absence of mature sperm and the reduction of spermatogenesis was evident in almost all the tubules. But testes of the tyrosine-fed rats treated with ascorbic acid in addition, showed normal process of spermatogenesis again.

The role of ascorbic acid on testicular activity is not clear. Many investigators[7,8] could not detect any change in testicular ascorbic acid when the organ was stimulated by gonadotropins. On the other hand, testicular degeneration has been noted in ascorbic acid deficiency[9,10]. GHOSH and GUHA[5] have previously reported a disturbance in ascorbic acid biosynthesis in rats on a high dietary intake of tyrosine. Testicular atrophy, as observed under similar conditions in the present experiment, might be due to lowered ascorbic acid status of the animals. It has also been reported that administration of ascorbic acid corrects the histochemical abnormalities of scorbutic testes[11]. DEB and CHATTERJEE[12] have observed that the same vitamin corrects testicular degeneration in alloxan diabetic rats. In the present study also, normal spermatogenic process appeared again on administration of ascorbic acid in the degenerated testes due to tyrosine feeding.

The mechanism of the above changes is not very definitely understood. SELYE[13], NOWELL[14] and VELARDO[15] have all observed that excessive ACTH release inhibited gonadotropin production from the anterior pituitary. DEB and BISWAS (unpublished) have recently noted hypertrophy of the adrenal gland together with a fall in sudanophilic lipids in the organ in rats fed a high dose of tyrosine, which was again corrected by ascorbic acid treatment. It might be possible that the role of high tyrosine level on male reproductive organ is mediated through its inhibitory effect on hypophysical gonadotrophic hormone release[16].

Résumé. La dégénération des testicules des rats nourris d'une dose toxique de «tyrosine» a été évitée par le traitement à l'acide ascorbique.

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Effect of Glucose Administration on Release of Prothrombin from Rat Liver Microsomes during Incubation

The function of vitamin K in the synthesis of prothrombin by the liver remains obscure. In a recent review, JOHNSON[1] concluded that vitamin K probably does not stimulate prothrombin formation from free amino acids, but may participate in the finalization of prothrombin from a precursor molecule, possibly through some role of the vitamin in electron transport. GOSWAMI and MUNRO[2] observed that microsomes prepared from rat liver and incubated in Krebs-Ringer solution show an increase in prothrombin activity. From the conditions of incubation, they concluded that this did not represent de novo protein formation but must have arisen from finalization of prothrombin. HILL, GAETANI and JOHNSON[3] confirmed that more prothrombin activity appears during incubation of microsomes, but concluded that this phenomenon is due to release of preformed prothrombin from the microsomes into the suspending medium. We now wish to report an experiment which suggests that the microsome may not play a purely passive role during incubation, since the rate of appearance of prothrombin activity can be influenced by pre-feeding of glucose to the donor animal.

Rat livers were homogenized in 10 Vol of 0.25 *M* sucrose and the heavy microsome fraction previously[2] found to generate prothrombin during incubation was isolated by removing the cell debris, mitochondria and nuclei by spinning for 10 min at 6500 *g*, followed by isolation of the heavy microsomes by centrifugation for 1 h at 18,000 *g*. This fraction was then resuspended in the same volume of sucrose as that used for homogenization. 2 ml of this suspension were incubated with 2 ml Krebs-Ringer bicarbonate buffer, pH 6.9, modified by omission of $CaCl_2$ which would interfere with prothrombin assay. The gas phase during incubation was 95% oxygen/5% CO_2. Samples were taken at intervals up to 3 h of incubation at 37° and assayed for prothrombin content by the ALLINGTON[4] modification of the one-stage procedure, which also measures the amount of factor VII present in the system. The results are expressed in units giving the prothrombin activity as a percentage of the prothrombin contained in a standard sample of rat plasma. The pro ein content of the microsome preparations was estimated by the LOWRY procedure[5].

Rats were fasted overnight and then received 3 g glucose orally. They were killed 2 h after feeding; control animals were kept fasting throughout. Liver from both

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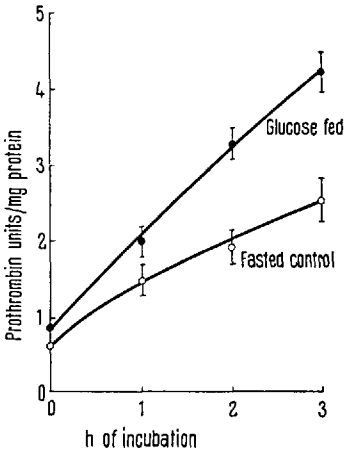
groups were homogenized and the isolated heavy microsomes were incubated as described above. The graph shows that prothrombin activity accumulated much more rapidly in the microsome preparations made from the carbohydrate-fed animals than in the microsome prepared from fasting animals. The vertical bars show the standard errors for variation in replicate experiments; this indicates that the rate of prothrombin accumulation in glucose-fed animals is significantly greater than in the control series.



Prothrombin activity of rat liver microsomes during incubation in Krebs-Ringer solution. The results are the mean of three experiments in which one group of rats were kept fasting and the other group received glucose 2 h before killing.

These findings suggest that intracellular energy supply may influence subsequent prothrombin release from microsomes. The factor involved was not identified. Although level of energy intake has a considerable effect on the ATP concentration in the liver[6], addition to the incubation of ATP, ADP, NAD and NADH at concentrations between 0.5 and 1.5 μmoles/ml of incubation medium inhibited prothrombin accumulation. Addition of NADP and NADPH did not affect prothrombin increment during incubation. Addition of dinitrophenol to the medium and incubation in the presence of nitrogen in place of oxygen had no effect for the first 2 h of incubation, and were somewhat inhibitory during the third hour.

Résumé. Les microsomes isolés du foie continuent à relâcher le prothrombin pendant l'incubation de façon désavantageuse pour la synthèse totale des protéines. L'accumulation est plus rapide quand les rats, soumis à un jeûne bref, étaient nourris de glucose avant le sacrifice.

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Attempts to Isolate C'_3 Activity from Pig Serum

In a previous investigation (Cavallo, Pontieri, and Imperato[1]) the ability of lysozyme to reconstitute the lytic activity of a reagent used for the titration of the activity of the third component of complement (C'_3), prepared with 'Liquoid' (sodium polyanethol sulphonate, Hoffmann-La Roche) treated serum was demonstrated.

Researches on the mechanism of this activity, played by either lysozyme or other basic proteins (Pontieri, Imperato, and Cavallo[2]), showed that the phenomenon was due to the formation of complexes between basic proteins and 'Liquoid' fixed in the reagent to the serum globulins of C'_3 activity furnished. The displacement of the inactivating agent from these determined the appearance of C'_3 activity which was inhibited in the reagent.

In the course of the above-mentioned work, it was observed that formation of insoluble complexes, which precipitate, takes place when 'Liquoid' is added to either guinea-pig or human serum. It seemed, therefore, of interest to determine whether or not C'_3 was carried down in the precipitate and, if such were the case, whether or not the recovery of C'_3 activity from the precipitate itself was possible.

Pig serum was selected as source of C'_3 because of its high content in this component of complement.

Figure 1 shows the degree of precipitate formation which takes place in pig serum when added with various amounts of 'Liquoid'. For all the further determinations 600 μg of 'Liquoid' were constantly added to each ml of pig serum.

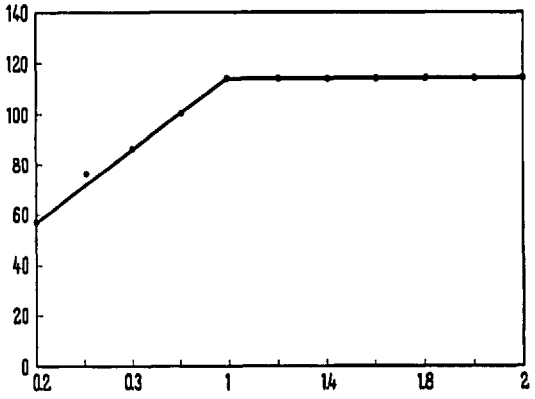

Fig. 1. Formation of insoluble complexes following addition of *'Liquoid'* to pig serum. Abscissae μg of *'Liquoid'*/ml pig serum. Ordinates: turbidimetric Klett Units.

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