10 min. With guanosine the rate is about one tenth of the rate with inosine.

Among the fractions obtained by differential centrifugation, the nuclei and mitochondria were devoid of nucleoside phosphorylase activity. The bulk of the enzyme was found in the soluble fraction, whereas the microsomes had an activity amounting to a few percents of the supernatant (on protein basis), activity which might well result from slight contamination. The intracellular distribution is thus in agreement with that found in other tissues[9].

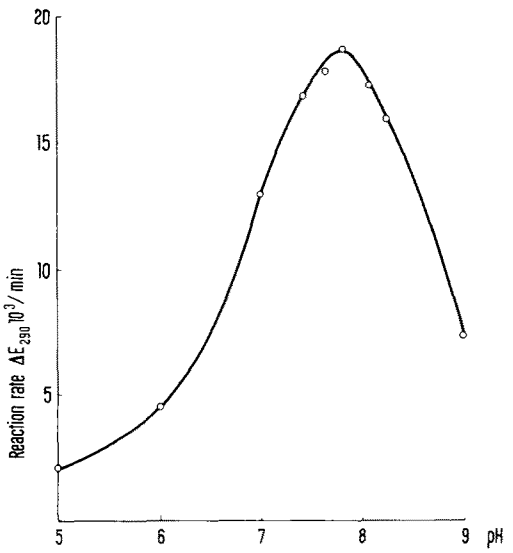

Fig. 2. Effect of pH on leukocyte nucleoside phosphorylase activity. Assay as in Figure 1. The pH 5 reaction mixture was buffered with citric acid maintaining the usual phosphate concentration. An effect of the citrate anion was ruled out by testing at neutral pH.

The pH-dependance is shown in Figure 2. Maximum activity is found at 7.8, which is close to findings on the beef liver enzyme[10]. Also the Michaelis-Menten constant for inosine ($3 \cdot 10^{-5} M$) is of the same order of magnitude with the liver[10] and the leukocyte enzyme.

If the nucleoside phosphorylase activity of white and red blood cells is compared, one finds (on recalculating the data by TSUBOI and HUDSON[11] on human erythrocytes) that the former have a 4-fold activity on a protein basis and an 8–10-fold activity on a cell basis.

Riassunto. Gli autori dimostrano la presenza di nucleoside fosforilasi nei granulociti neutrofili di essudato peritoneale sterile di cavia. L'attività enzimatica è distribuita quasi totalmente nella frazione solubile, presenta attività 10 volte più elevata sull'inosina che sulla guanosina, ha pH ottimale 7,8 e costante di Michaelis-Menten per l'inosina uguale a $3 \cdot 10^{-5} M$.

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Centro di Biochimica dei Tumori «Cassa di Risparmio di Genova» presso l'Istituto di Chimica Biologica dell'Università di Genova (Italy), March 15, 1962.

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Maltase Activity in Human Semen

It has been demonstrated that mammalian spermatozoa are capable of utilising glucose, fructose, mannose, maltose and glycogen for the maintainance of sperm motility and metabolism (KOELLIKER[1] and MACLEOD[2]). Prior to their utilisation, it is necessary that disaccharides should be converted to monosaccharides. The enzymes responsible for such conversions have not been studied in detail so far. KARASSIK[3] has reported the presence of amylase in dog and human semen. The observation of LANE-ROBERTS et al.[4] that human semen contains an unidentified substance which on addition of amylase gets converted to glucose indicates that glycogen may also be present. It seemed likely that, as in the case of salivary amylase, seminal amylase could then bring about the conversion of seminal glycogen to maltose. It was therefore thought possible that degradation of maltose to glucose would be brought about by an enzyme similar to intestinal maltase. With these observations in view, studies were undertaken to detect and estimate the maltase activity of human and other animal semen.

Semen samples were obtained from fertile donors. Buffalo semen was obtained from the Maharastra Government milk colony. Rabbit semen was collected by means of an artificial vagina and cock semen by abdominal massage. Maltase activity was estimated in terms of glucose liberated. The glucose was estimated by the paper chromatographic method of SHETH and RAO[5]. The spray reagents used for the detection of sugars were the same as described earlier (SHETH and RAO[6]). Protein concentration was determined by the method of LOWRY et al.[7]. All enzyme studies were carried out using veronal acetate phosphate universal buffer.

The maltase activity of semen was estimated as follows. To 0.5 ml of semen were added equal volumes of veronal buffer pH 5.5 and $0.14 M$ maltose. Appropriate control mixtures were also prepared, one without the seminal plasma and the other without the substrate. The mixtures were incubated at 37°C for 24 h. In the reaction as well as the control mixtures, 2 to 3 drops of toluene were added before incubation. After incubation, the sugars present in the mixtures were identified and estimated according to the method of SHETH and RAO[5,6].

The results indicated that two bands developed from the point spotted with the digestion mixture. One of these bands was due to maltase and the second band was identified to be that of glucose. The formation of glucose from maltose indicated that maltase was present in dia-

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lyzed seminal plasma. Spermatozoa washed twice with saline did not show any maltase activity.

Microbiological tests carried out with the control and reaction mixtures indicated that no microorganisms had grown during the period of incubation.

The maltase activity of seminal plasma was maximal at pH 4.5 to 5.0. The enzyme activity rapidly diminished on heating above 40°C. Heating at 55°C destroyed nearly 50% of the activity and at 70°C only about 15% of the original activity was left. The results indicate seminal maltase may also have three types of maltase with different degree of heat stability as reported for hog intestinal maltase by DAHLQUIST[8].

Tab. I. Maltase activity of seminal plasma of men, cock, bull, and rabbit

Species	Mean of 5 samples mg/ml glucose liberated	S.E. of Mean
1. Human	10.76	± 1.0
2. Cock	5.42	± 0.41
3. Bull	5.02	± 0.84
4. Rabbit	1.78	± 0.33

Tab. II. The activity of human seminal plasma with different sugar substrates

0.14 *M* of sugar substrate	Glucose formed mg/ml	0.14 *M* of sugar substrate	Glucose formed mg/ml
1. Maltose	12.7	6. Melibiose	1.2
2. Turanose	10.2	7. Raffinose	1.1
3. Trehalose	2.8	8. Genetiobiose	0.9
4. Sucrose	2.3	9. Cellobiose	0.45
5. Lactose	1.5		

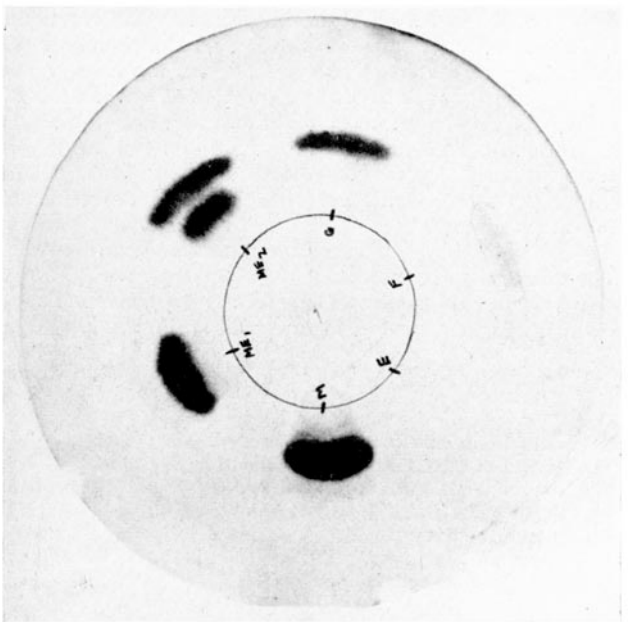

Fig. 1. The effect of human semen on the maltose substrate: ME_1-Reaction mixture containing 0.5 ml of maltose (0.140 *M*), dialysed semen and buffer. ME_2: Reaction mixture after 24 h. E: Dialysed semen and buffer. M: maltose and buffer. G: glucose. F: fructose.

Semen samples of bull, rabbit, cock and human were collected as already described. The seminal plasma were separated by centrifugation and dialysed. The protein was determined in dialysed seminal plasma and suitable dilutions were then made with distilled water so that they contained equal amount of protein (200 µg/ml). The maltase activity was determined as described before. The results as indicated in Table I show that the human seminal plasma has the maximum maltase activity.

The enzyme activity of seminal plasma with different sugar substrates was studied to find out the relative activity of seminal plasma with the different sugars as compared to maltose. The different sugar substrates were used in 0.14 *M* concentration. The glucose liberated was measured as already described. Results shown in Table II indicate that seminal plasma exhibited maximum activity towards maltose.

MACLEOD[2] showed that human spermatozoa were capable of utilising maltose. The results reported here did not indicate that human spermatozoa possessed maltose-hydrolysing ability. This observation is in agreement with

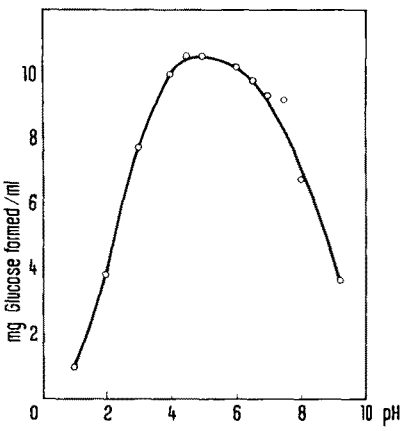

Fig. 2. The effect of pH on the maltase activity of human seminal plasma.

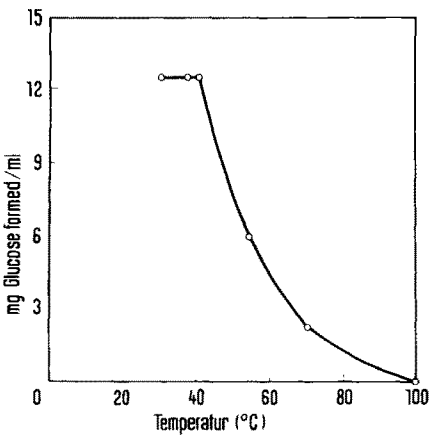

Fig. 3. The effect of temperature on the maltase activity of human seminal plasma.

[8] A. DAHLQUIST, Acta chem. scand. *13*, 945 (1959).

the observation of MOORE and MAYER[9] who reported that ram spermatozoa did not possess the enzymes necessary for the hydrolysis of α- and β-glucosidic linkages. The possible explanation of the results obtained by MACLEOD could be that unwashed spermatozoa were used in the studies reported by him. It is known that by mere centrifugation spermatozoa cannot be completely freed from seminal plasma. So the maltase present in the seminal plasma adhering to unwashed spermatozoa must have hydrolysed the maltose. We found that washing the spermatozoa twice with saline deprived them of their maltase activity indicating that seminal plasma was removed by this treatment.

The detection of maltase activity in human semen is of considerable importance since we have observed that human semen contains appreciable amounts of carbohydrate as glycogen from 0.14 to 5.5 mg/ml. Glycogen would be converted to maltose by amylase which is known to be present in human semen (KARASSIK[3]). Further hydrolysis of maltose by maltase would result in the formation of utilisable sugar, glucose. Thus it seems that maltase may play an important role in the glycolytic metabolism of human spermatozoa. Further work on the purification and properties of seminal maltase will be reported elsewhere.

Résumé. Nous avons étudié l'activité de la maltase dans la semence humaine en utilisant la technique de la chromatographie sur papier. L'activité de la maltase est exprimée en mg de glucose liberé du substrat de maltose. L'activité de la maltase est spécialement associée avec le plasma séminal. Elle est maximale au pH 5.0. Nous avons aussi étudié les activités de la maltase dans des plasma séminaux des êtres humains, des bœufs, des coqs et des lapins. Nous avons trouvé que l'activité de la maltase est la plus haute chez l'homme. En comparaison avec les autres substrats de sucre, l'activité du plasma séminal se montre plus haute dans la maltose.

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Reproductive Physiology Unit, Indian Cancer Research Centre, Parel, Bombay (India), April 16, 1962.

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[10] *Acknowledgment.* It is a pleasure to acknowledge the help and guidance of Dr. V. R. KHANOLKAR, Director, Indian Cancer Research Centre, during the course of these studies.

Spécificité de l'antigène tumoral décelé dans le foie de rat porteur d'une tumeur de Walker[1]

Certains auteurs ont voulu associer l'antigène tumoral, que nous avions détecté dans le foie du rat porteur de la tumeur de Walker[2-5], au facteur protéique du sérum que DARCY[6] et HEIM[7] ont découvert dans le sang de rats soit nouveau-nés, soit en gestation, soit porteurs de tumeurs. Ce facteur sérique relié à la reproduction et à la synthèse tissulaire n'est évidemment pas spécifique au cancer.

Aussi, il nous a semblé important de faire des études systématiques de comparaison du comportement antigénique du foie provenant d'animaux dans différents états biologiques et pathologiques; soit du foie de rats embryonnaires, nouveau-nés, en gestation et hépatectomisés (conditions biologiques) et porteurs de la greffe tumorale, d'une arthrite à la formaline et d'inflammation aigüe (conditions pathologiques).

Matériel et Méthodes. 28 rats mâles et femelles de souche Fisher inbred 344 provenant de l'élevage Charles River, Brooklyne, Mass., U.S.A., ont été distribués également en 7 groupes suivants dont chacun représente un état physiologique ou pathologique déterminé: (1) rats à l'état embryonnaire; (2) rats nouveau-nés; (3) rattes gestantes; (4) rats partiellement hépatectomisés depuis 72 h; (5) rats porteurs d'une tumeur de Walker transplantée par voie intramusculaire depuis 15 jours; (6) rats atteints de l'arthrite à la formaline, provoquée expérimentalement depuis 6 jours; (7) rats ayant reçu une injection d'huile de croton dans une poche d'air dorsale depuis 6 jours et qui sont alors marqués par une inflammation aigüe.

Tous les animaux sont sacrifiés par décapitation. Le foie est soigneusement perfusé avec une quantité abondante de sérum physiologique jusqu'à disparition complète des traces de sang. La pesée faite, on procède ensuite à une homogénéisation automatique à une vitesse de 24000 tours à la minute, à l'aide d'une solution saline isotonique de NaCl dans la proportion 1:2 (1 g de tissu pour 2 ml de solution saline). L'homogénéisat est ensuite soumis à une centrifugation réfrigérée à 60000 *g* pour 1 h. Le surnageant ainsi obtenu constitue l'extrait hépatique soluble dont nous déterminons immédiatement la concentration en azote protéique par microkjeldahl. Nous rajustons alors la concentration protéique, si nécessaire, à l'aide de la solution saline isotonique, de façon telle qu'elle soit la même pour tous les extraits hépatiques.

La fabrication de l'immunsérum antitumeur chez le lapin se produit par suite des injections alternatives, par voie intraveineuse, intramusculaire et intrapéritonéale, des extraits lyophilisés d'une tumeur de Walker suivant les détails indiqués dans une publication antérieure[2]. Pour débarasser les anticorps non spécifiques, formés à partir des impuretés dans l'extrait tumoral, nous avons procédé à une absorption en série de l'immunsérum, d'après la technique de HEIDELBERGER et KENDALL[8], à l'aide des petites quantités d'extrait soluble de foie normal. L'immunsérum ainsi purifié est utilisé dans la détection de l'antigène tumoral.

Nous nous sommes servi de l'immunoéléctrophorèse telle que décrite par GRABAR et WILLIAMS[9], pour étudier la spécificité et la nature de l'antigène tumoral décelé dans le foie de rat porteur d'une tumeur de Walker.

[1] Travail subventionné par l'Institut du Cancer du Canada.

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