No. 1 paper. Concurrently, 14 amino-acids (Merck) were compared on the same paper. For these Merck amino-acids the solvent was 10 c.c. *tert.*butanol, 15 c.c. 98 per cent formic acid, 15 c.c. water. The chromatogram was examined with ninhydrin reagent after 20 h.

We have found for each of the three species of tobacco seeds six amino-acids, characterized as leucine, valine, alanine, glutamic acid, aspartic acid and arginine. An uncertain spot appeared in the place for lysine, but to be certain, this was repeated using a solvent of acetic acid, *n*-butanol and water. The seventh point repeated itself for lysine.

The lysine and valine spot for the *N. trabzant* seed was very weak. The lysine spots for *N. tikolak* and *N. basmacerus* were also weak.

To confirm the foregoing results, a two-dimensional chromatogram was run. In the first dimension, the solvent was *tert.*butanol, formic acid and water, and in the second dimension the solvent was 400 g phenol, 100 c.c. water in an atmosphere of ammonia 20 c.c. 0/3 per cent ammonia solution. The result of the two-dimensional chromatogram showed the existence of six amino-acids: leucine, valine, arginine, glutamic acid, aspartic acid and alanine. Lysine did not appear in this chromatogram perhaps because its quantity was too scarce to be stained sufficiently for detection.

The results show that at least 6 amino-acids do exist in the free state in mature tobacco seeds of some species. Further, 4 or 5 of these free amino-acids are among the essential amino-acids for animals, for example, rats.

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[1] Abdoh, Y., and Taufel, K., *Z. Untersuch. Lebensmittel*, **84**, 328 (1942).
[2] Il'in, G. S., and Lovkova, M., jun., *Biekhimija U.S.S.R.*, **24–5**, 838 (1959).

Intermediary Metabolism in Bull Semen

We have examined the incorporation of radiocarbon from [^{14}C] glucose, [2-^{14}C] acetate and [1 : 4-$^{14}C_2$] succinate into the soluble metabolic intermediates of bull semen. Samples (50 μl.) of the semen, used within 6 h of collection, were mixed with 50 μl. of a solution containing 0·1 mM adenosine triphosphate, 2 mM magnesium sulphate, 10 mM potassium chloride and 1 μc. of the labelled substrate dissolved in 0·1 M potassium phosphate buffer, *p*H 7·4. The mixtures were incubated aerobically at 37° C for 30 min. At the end of the incubation period the enzyme systems were inactivated by the addition of 400 μl. of boiling ethanol and the mixtures centrifuged at 500*g*. The radioactive substances present in the supernatants were separated by two-dimensional paper chromatography, visualized by autoradiography and the carbon-14 measured by the techniques described by Smith and Moses[1].

The results (Table 1) show that whole bull semen utilized 99 per cent of the labelled glucose and that radiocarbon was incorporated mainly into hexose phosphates and lactate. Redenz[2] has shown that bull spermatozoa are able to glycolyse various sugars, including glucose, to lactate, and it has become an established fact that the metabolism of spermatozoa in several mammalian species is predominantly of a glycolytic character[3]. The presence of radioactivity in fructose is explicable because the initial product of glycolysis, glucose-6-phosphate, is readily converted by spermatozoal phosphohexose isomerase to fructose-6-phosphate, which may form fructose as a result of the action of phosphatase enzymes in the seminal plasma.

Table 1. Metabolism of Labelled Substrates by Bull Semen
Results given as ^{14}C (counts per min × 10^{-2}) incorporated into each soluble intermediate

[^{14}C] Glucose		[2-^{14}C] Acetate		[1:4-$^{14}C_2$] Succinate	
Intermediate	^{14}C	Intermediate	^{14}C	Intermediate	^{14}C
Hexose phosphates	29	Lactate	90	Fumarate	113
Fructose	2	Alanine	1	Malate	330
Lactate	16	Glutamate	3	Citrate	1
% of labelled		Glutamine	9	Aspartate	1
glucose utilized	99	Citrate	4	Glutamate	1
		Succinate	1	% of labelled	
		Fumarate	2	succinate utilized	67
		Malate	5		

Most of the incorporated isotope from the labelled acetate was found in lactate which was presumably formed via pyruvate and acetylocoenzyme *A*. The preponderance of labelled lactate over labelled alanine in both the radioactive glucose and acetate experiments suggested that transamination of pyruvate does not occur to any appreciable extent in bull semen. In the acetate and succinate experiments only small amounts of radiocarbon were incorporated into aspartate and glutamate, and Bhargava, Bishop and Work[4] have shown that the intracellular amino-acid pools of bull spermatozoa are low compared with those in other mammalian tissues. However, the presence of radioactive glutamine in the acetate experiments shows that bull semen possesses glutamate synthetase activity. Succinic acid has been reported to have little effect on the oxygen uptake of intact mammalian spermatozoa[3]. In the work recorded here, the bull semen was found to utilize 67 per cent of the labelled succinate; but the incorporated radiocarbon accumulated in fumarate and malate, with only trace amounts in aspartate, citrate and glutamate. These results suggest that bull semen possesses appreciable succinate dehydrogenase activity but only a very limited capacity to metabolize malate.

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[1] Smith, M. J. H., and Moses, V., *Biochem. J.*, **76**, 579 (1960).
[2] Redenz, E., *Biochem. Z.*, **257**, 234 (1933).
[3] Mann, T., *The Biochemistry of Semen* (London: Methuen and Co., Ltd., 1954).
[4] Bhargava, P. M., Bishop, M. W. H., and Work, T. S., *Biochem. J.*, **73**, 242 (1959).

A Rapid Method of Disk Electrophoresis

Disk electrophoresis, as originated by Ornstein and Davis[1], provides a delicate analytical technique for the resolution of protein mixtures, but it requires nearly 2 h to complete a run. The formation of two acrylamide gels which merely prevent convection and take no direct part in the separation occupies some forty minutes. Barka avoided this step by simply layering the proteins in an appropriate buffer over the running gel[2]. Reisfeld[3] added sucrose to the sample mixture so that the density gradient diminished diffusion, but it has been reported that this manœuvre necessitates a reduced voltage during electrophoresis[4].

This communication describes a simple general procedure which gives a result in about 70 min. This same procedure enables electropherograms of the plasma proteins (as anions) to be obtained from capillary blood and, finally, some other applications are outlined.

A typical plasma protein electropherogram from equine whole blood is illustrated in Fig. 1*a*. To achieve this, the 'running gel' (*p*H 9·1) was formed in a glass tube exactly as described by Ornstein and Davis, but immediately before use it was overlaid with a 1-cm column of a buffer–