concentrations of barbiturates necessary to inhibit phosphorylation and those necessary to inhibit respiration.

McElroy(12) proposed that narcotics act not by merely depressing the respiratory rate of nervous tissue but by inhibiting some energy-yielding process, notably phosphate bond turnover, thereby depressing or abolishing neuronal activity. A recent paper by Westfall(15) indicates that small doses of barbiturates produce a small increase in the oxygen consumption of rat cerebral slices. Our data show a similar effect with particulate preparations (Fig. 1). Thus, barbiturates are no exception to the suggestion of Lardy and Elvehjem(16) that compounds which speed up the metabolic rate at the same time decrease the available energy by allowing respiration to take place without phosphorylation.

Further evidence that the mere depression of the respiration of the brain is inadequate to explain the pharmacologic effects of these compounds is shown by comparing the metabolic deficiencies of the brain in hypoglycemic coma and in thiopental anesthesia(17). In the former, metabolic rate and nervous activity fall equally; while in the latter, the fall in metabolic rate is small when the narcosis is deep. Larrabee, *et al.*(18) have also shown in studies on excised superior cervical ganglia that nervous activity may be depressed without a proportionate fall in oxygen consumption. All of which indicates that barbiturates may depress nervous activity *in vivo* by uncoupling oxidation from phosphorylation.

Summary. It has been shown that thiopental, pentobarbital and amobarbital lower the phosphate : oxygen uptake ratio obtained in particulate tissue preparations from liver and brain respiring on a pyruvate substrate. This depression is obtained at concentrations of the drugs which have only a slight effect on the oxygen uptake of the preparations and which approximate those which are necessary *in vivo* to produce surgical anesthesia. It is postulated that such an uncoupling of phosphorylation from oxidation may be one of the ways in which barbiturates act to produce anesthesia.

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Chemical Composition of Bovine Spermatozoa. (18675)

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Information on the chemical composition of spermatozoa is fundamental to a better understanding of the functional significance of the male gamete in the fertilization process as well as its contribution to subsequent developmental processes. There have been in the past several investigations dealing with various aspects of this problem. The work of Kossel(1) on the protamines of fish sperm in his researches dealing with the structure of basic proteins is an example. More recent-

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ly, Zittle and O'Dell(2) determined the methionine content of bovine spermatozoa and Sarkar *et al.*(3) reported values on several amino acids found in sperm of the same species.

Data are presented here on 17 amino acids present in bovine spermatozoa as well as on the total nitrogen, total lipid, total phosphorus, and ash content.

Materials. Preparation of spermatozoa. Semen samples were collected at weekly intervals from 7 bulls used for artificial insemination investigations at Iowa State College. Microscopic examination of the semen samples used in this work indicated that little, if any, contaminating material such as tissue debris or leucocytes was present. Each ejaculate ranging from 3 to 7 ml was diluted with two volumes of buffered 0.9 per cent NaCl (pH 6.8) and centrifuged. The supernatant fluid was aspirated and the spermatozoa were resuspended in buffered saline and allowed to stand 10-15 minutes before centrifuging. The spermatozoa were washed 8 to 10 times in a similar manner with buffered saline and finally washed once with distilled water. The samples of the washed spermatozoa were dried at 105°C, composited, and stored for chemical analyses and microbiological assays. Approximately 700 mg of material were prepared for use in this work.

Methods of analysis. Total nitrogen was determined by the micro-Kjeldahl method, total lipid by ether extraction of moisture-free material, and ash by ignition after adding sulphuric acid. Phosphorus was determined gravimetrically according to a procedure described by Elek(4) and Elek and Hill(5). The amino acids were determined by microbiological assays and the response of the test organism was determined titrimetrically. Acid hydrolysis was used in the preparation of dried bovine spermatozoa for the amino acid assays except for tyrosine and tryptophan where alkaline hydrolysis was employed. Leucine, isoleucine, and valine were determined by use of *Lactobacillus arabinosus* 17-5 by a modification of the procedure developed by Shankman(6). The media as used in these determinations contained all of the recognized nonessential amino acids and 0.6% ammonium chloride, 2% dextrose, and 1.2% sodium acetate. Tryptophan was determined in a similar medium but ammonium chloride was omitted. Glutamic acid was assayed according to the method of Dunn *et al.*(7) and ammonium chloride was added to the medium. *Leuconostoc mesenteroides* P-60 was used in determining lysine, histidine and phenylalanine employing methods described by Dunn *et al.*(8-11). Glycine was determined according to Shankman *et al.* (12,13) using the same organism. An unpublished procedure with *L. mesenteroides* P-60 by Shankman was used for the assay of serine, proline, and aspartic acid. The amino acid level in these media was 1.33 times that employed in the previously indicated lysine assay. Incubation time was 3 days. Hydroxyproline and norvaline were omitted from the media used in determining these amino acids. Cystine was assayed with *L. mesenteroides* P-60 according to Camien and Dunn (14). Hydrolyses with 1:1 hydrochloric-formic acid under reflux and with 10% hydrochloric acid in a sealed tube gave identical

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data for this amino acid.

Lactobacillus fermenti 36 was used for the threonine and methionine assays and the procedure of Dunn *et al.*(15-17) was followed. Threonine was also assayed with *Streptococcus faecalis* according to Stokes *et al.*(18). Arginine and tyrosine were determined with *Lactobacillus casei* according to Shankman *et al.*(19) and this medium was modified to contain 2% dextrose, 1.2% sodium acetate, and all the amino acids indicated except hydroxyproline, norleucine, and norvaline. Two assays, at 2 different dilutions of hydrolyzate, were made for each amino acid. A control assay on casein hydrolyzate was also made with each amino acid determination and the entire assay was repeated if the results obtained did not agree with accepted values in the literature for casein. A common complete vitamin mixture was used for all assays. Furthermore, in evaluating the analytical data presented here, it is necessary to stress that amino acid assays on whole sperm do not differentiate between free amino acids which may be present in the cell and those constituting protein molecules.

Results. The total nitrogen content of bovine spermatozoa was found to be 16.7% on a moisture- and lipid-free basis. Analyses of dry sperm showed a total lipid content of 1.2%, ash 1.1%, and total phosphorus 2.3%. The results of the amino acid assays are presented in Table I.

TABLE I. Amino Acid Composition of Bovine Spermatozoa. Results expressed in % on a moisture, ash, and lipid-free basis.

Amino acid	Assay 1 %	Assay 2 %	Avg %
Arginine	15.4	14.6	15.0
Aspartic acid	5.0	5.0	5.0
Cystine	2.8	2.7	2.8
Glutamic acid	6.2	6.5	6.4
Glycine	3.5	3.6	3.6
Histidine	1.7	1.7	1.7
Isoleucine	2.4	2.5	2.5
Leucine	4.6	4.4	4.5
Lysine	4.3	4.5	4.4
Methionine	1.5	1.4	1.5
Phenylalanine	2.6	2.7	2.7
Proline	3.1	3.1	3.1
Serine	4.4	4.5	4.5
Threonine	3.3	3.1	3.2
Tryptophan	1.0	1.0	1.0
Tyrosine	4.6	3.9	4.3
Valine	3.2	3.3	3.3

Discussion. Dried bovine spermatozoa are high in total nitrogen as indicated by the data presented here. The average value of 16.7% is somewhat lower than the value obtained by Sarker *et al.*(3). Arginine is the most abundant amino acid present in the sperm cell and the value of 15.0% obtained in this work is much lower than the 25.47% reported by Sarkar *et al.*(3). Furthermore, the histidine, lysine, tryptophan, phenylalanine, leucine, isoleucine, and glutamic acid data presented in Table I are lower than those presented by Sarkar *et al.*(3). However, there is good agreement between both sets of data for methionine, threonine, and valine. Zittle and O'Dell(2) have reported the methionine content of bovine sperm as 1.92% on a moisture- and lipid-free basis.

The bovine spermatozoon may be characterized as a cell with a small amount of cytoplasm and a large nucleus and, as a result, this cell is relatively high in nucleoprotein. It is of interest that tryptophan occurs in small amounts in this cell as indicated by the data in Table I.

It was found by Melampy(20) in a cytochemical study of the amino acids of the chicken erythrocyte that the tryptophan content of the nuclei was low. Mirsky and Ris (21) in their work on the composition of chromosomes have found a small amount of tryptophan in the histones of fowl erythrocytes. Methionine and histidine are also present in relatively low concentrations in the bovine sperm cell.

Summary. The chemical composition of

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bovine spermatozoa has been determined. Data are presented including total N, total lipid, ash, and total phosphorus. Seventeen amino acids were determined by microbiological assays.

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One Stage Method of Hepatectomy in the Dog.* (18676)

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Complete removal of the liver from dogs has been achieved by various methods(1-3). The following new, one-stage method has given satisfactory results to the authors and can be executed without great difficulty. The abdomen having been widely opened, and the incision kept apart with a self retaining retractor, the gastro-hepatic omentum is cut, after the portal vein has been freed from all other structures. The portal vein is cut against the liver, introduced into a small metallic tube according to Queirolo's method(4) and implanted sideways into the vena cava above the renal veins. The liver is then severed from the abdominal wall. When this is done, a strong thread is placed around the vena cava against the diaphragm in order to prevent the reflux of the blood from the heart. The flow of the blood to the liver has been previously stopped by 2 clamps placed one on the vena cava and the other on the portal vein upstream from the portal-cava fistula. Then a veinous graft which is to replace the intra-hepatic part of the vena cava is put into position. The graft is the intra-thoracic part of the vena cava of another dog used in a previous operation; it has been kept at 0°C in Tyrode solution to which penicillin and a sulfamide have been added. After the vena cava of the dog has been cut transversely under the liver and against it, the inferior part of the graft is inserted into the caudal segment of the vein and fixed above the portal-cava fistula. The other end is introduced into the intra-hepatic part of the vena cava and pushed upwards until it finds its place between the top edge of the liver and the diaphragm. The ligature lying behind the liver is then firmly tied on it. The clamps placed on the portal vein and the vena cava being taken away, one proceeds to the extirpation of the liver after having split the intra-hepatic part of the vena cava from the lower end to the top.

Dogs which have undergone this operation, performed with an aseptic technic, survived for 12-14 hours. Death occurs from circulatory collapse. To restore the vascular tonus, we resorted to the use of a hydrosoluble derivative of desoxycorticosterone added in sufficient quantity to the glucose solution, which is injected, drop by drop, into a vein. The survival of 3 dogs which received this treatment, was respectively 16, 26 and 31 hours.

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* A detailed description of the procedure is given in English in a paper to be published in *Arch. Internat. de Pharmacodynamie et de Thérapie.*

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