TABLE III. Metabolism of Pyruvate in Spermatozoa. Washed bull spermatozoa incubated in phosphate saline at 37°; gas, air. Fumarate (.002 M) and fluoride (.02 M) in all flasks. Pyruvate (.01 M) unless otherwise stated. DNP, 10^{-4} M. Volume, 4 ml.

Type of metabolic response	Incubation period, min	Sperm count /flask × 10^8	Additions	Z (μl/10^8 spermatozoa/hr) O_2	Pyruvate	Lactic acid	Net pyruvate	Ratio O_2/net pyruvate
A	70	7.92	0	− 4.2	—	—	—	—
			Pyruvate (.005 M)	− 5.2	−25.3	+16.3	−9	.6
			" " , DNP	−11.8	− 7.7	+ 2.4	−5.3	2.2
B	90	10.2	0	− 8.6	—	—	—	—
			Pyruvate	−14.1	−30.5	+23.4	−7.1	2
			" , DNP	−16	−12.5	+ 4.4	−8.1	2
C	90	11.7	0	− 4.9	—	—	—	—
			Pyruvate	−11.5	−38.7	+29.6	−9.1	1.3
			" , DNP	−14.9	− 7.4	+ 1.2	−6.2	2.4
D	120	9.1	0	− 6	—	—	—	—
			Pyruvate	− 9.1	−12.8	+ 9	−3.8	2.4
			" , DNP	− 8.2	− 7	+ 3.6	−3.4	2.4

semen of poor fertilizing capacity (types B and D) which, because of its good appearance and motility, might otherwise be considered satisfactory. The test may also be used for a periodic check on the quality of semen produced by bulls of fluctuating fertility. 3. Fructose, glucose, or lactate are not suitable as substrates since their utilization is in all cases increased by DNP.

Summary. Bull spermatozoa were graded according to their metabolic behavior on incubation with pyruvate, fluoride and 2:4-dinitrophenol. A correlation was shown between the type of response of the samples *in vitro* and the fertility of the donors in the artificial insemination of cattle.

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Method for Determination of Vitamin A in Stool.* (19603)

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Studies conducted in this laboratory on the metabolism of vit. A required a method for the quantitative determination of the concentration of this vitamin in stool. A search of the literature failed to reveal a satisfactory method. The procedure reported by Wendt (1) involves drying the stool with anhydrous sodium sulfate and subsequent extraction of vit. A with alcohol. This procedure is time consuming and may destroy some of the vitamin. Wendt did not report the degree of re-

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covery of vit. A by his method. The following quantitative method for determination of vit. A in stool was therefore developed.

The method depends upon preparation of a uniform suspension utilizing a magnetic stirrer. Samples of the suspension are then digested with alkali. A mixture of xylene and kerosene is used to extract the vit. A from the digested suspension again using the magnetic stirrer. The extracted vit. A is then determined by ultraviolet destruction as in the Bessey *et al.* method(2).

Method. The entire stool specimen is weighed and transferred into an Erlenmeyer flask. A weighed amount of 6% alcoholic potassium hydroxide is added. The amount of this potassium hydroxide to be added is estimated by experience according to the consistency of the original stool. If the stool is watery, considerably less of the potassium hydroxide solution is needed than if it is more solid. This amount may vary from one-third to an equal quantity of the weight of the stool. A magnetic stirring bar is placed into the flask and the whole placed on the magnetic stirrer until there is a uniform suspension. Between 2 to 3 g of this suspension are then weighed into a test tube of approximately 20 ml size. The volume in the test tube is made up to about 5 ml by addition of 6% alcoholic potassium hydroxide. The test tube is then covered with a glass marble and placed in a water bath at 70°C for 6 to 8 hours. (If the digestion cannot be finished the same day, the test tube may be kept in a refrigerator overnight.) The digested mixture is then filtered (Whatman No. 40 filter paper) by gravity into a 10 ml volumetric flask; the filter is washed with 6% alcoholic potassium hydroxide solution until the volume in the volumetric flask is 10 ml. To 2 ml of this alkaline filtrate in a 50 ml Erlenmeyer flask, add 2 ml of a xylene-kerosene (1:1) mixture. The magnetic bar is placed into this flask and the mixture is agitated vigorously for 10 minutes. The mixture is then allowed to stand until the layers separate. From the supernatant xylene-kerosene extract, 2 aliquots of 0.08 ml are drawn. The extinction coefficients of the samples are read in a Model DU Beckman spectrophotometer at 328 mμ; the samples are then irradiated and the extinction coefficients are again read at 328 mμ in the manner described by Bessey *et al.*(2). The average of the results obtained from the 2 aliquots is reported. In case the vit. A content of the stool proves to be too low to read in the spectrophotometer, the determination should be repeated by extracting 2 ml of the alkaline filtrate with 1 ml of the xylene-kerosene mixture as stated above.

Calculation. The vit. A values are obtained by the following calculation: $\frac{E_1 - E_2}{v - w} \times 637 =$ μg vit. A in 1 g stool, where E_1 is the extinction coefficient at 328 mμ before irradiation, E_2 is the extinction coefficient after irradiation, v is the quotient of 100 divided by the volume to which the filtrate was made up after saponification, w is the weight of stool sample in g; 637 is Bessey's factor.

Results. Suspensions of 10 different stools with alkali were prepared as in the first step described above. Two samples were removed from each suspension and analyzed for vit. A. To the remainder, a known amount of vit. A was added. The suspension with added vit. A† was stirred thoroughly again with the magnetic stirrer for at least 10 minutes. Two more samples were removed and the vit. A concentration was determined on each sample.

Table I shows the duplication of results which may be expected. The mean percentage difference between 2 determinations on the same stool suspension was 9.1% with a range of 0 to 18% (stand. dev. 5.2%). Table I also shows the per cent of recovery of known amounts of added vit. A. These recovery tests show that the mean per cent recovery is 6.25 (range 1 to 18) and that the standard error of a single determination (stand. dev.) is 7.5%.

It was found that if stool samples are kept in tightly closed "ointment type" jars in a refrigerator, the vit. A. concentrations do not change for at least 72 hours.

Discussion. The first requirement in a method for the accurate determination of vit. A concentration in stool is the preparation of a homogeneous stool sample. Drying of the

† The vitamins used in this study were kindly supplied by the Hoffmann-La Roche Co., Nutley, N. J.

TABLE I. Recovery of Added Vitamin A from Stool.

Human subject	Vit. A, μg/g stool: Before addition of vit. A	Mean	Vit. A added	After addition of vit. A	Mean	% recovery of vit. A
T.	2.05 2.28	2.17	3.5	6.30 6.12	6.19	109.1
V.	1.80 1.76	1.78	2.26	4.00 4.00	4.00	101
C.	1.59 1.65	1.62	2.81	4.15 4.6	4.36	101.4
J.S.	5.2 5.9	5.5	22.2	30.8 34.5	32.7	118
P.	5.8 6.9	6.35	16.7	23.9 26.9	25.4	110
H.	5.8 5.1	5.45	7.85	14.3 13.6	12.5	99
F.T.	.75 .90	.83	5.00	5.2 5.9	5.6	96
J.C.	2.26 2.00	2.13	28.6	32.4 29.8	31.1	101.3
M.	0 0	0	14.9	16.1 17.6	16.9	113.4
S.M.	3.3 2.9	3.1	15.6	17.3 18.8	18.1	96.7

stool and sampling of the dry powdered mixture is not satisfactory since drying of stool requires considerable time, and since vit. A is not stable to heat and oxygen. Preparation of a homogeneous sample from fresh stool was a difficult problem until it was solved by using a magnetic stirrer as described above. The size of the stirring magnet can be varied according to the amount of stool so that even small quantities may be used. Thus well blended mixtures can be prepared from which uniform samples can be taken.

Summary. 1. A method is described for the quantitative determination of vit. A in stool. 2. Alcoholic alkali is added to fresh stool and a uniform mixture prepared by using a magnetic stirrer. The method of extraction is described in detail. The standard percentage deviation of the difference between 2 determinations on the same stool was shown to be 5.2% and the standard deviation of the per cent recovery of added vit. A is 7.5%.

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Protogen and its Relation to Ketoacids in Metabolism of Tetrahymena.* (19604)

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The function of protogen (thioctic acid, Factor II, acetate factor, pyruvate oxidation factor, α-lipoic acid(1-6)) in the metabolism of lactic acid bacteria(4,5,7) appears to be concerned solely with the oxidation of pyruvate with the simultaneous production of acetate. In the case of *Tetrahymena geleii*, however, it has been shown that acetate

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