J. Reprod. Fert. (1968) **17**, 563–566

FRUCTOSE, LACTIC ACID AND CITRIC ACID CONTENT OF THE SEMEN OF ELEVEN SUBHUMAN PRIMATE SPECIES AND OF MAN

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(*Received 28th June* 1968)

Comparative studies of semen characteristics in subhuman primate species should be of value in the selection of appropriate species for experiments in reproduction. In addition, basic information obtained in such studies may be helpful for the successful preservation of frozen semen and insemination. These techniques will probably be essential in the maintenance of large primate colonies. Finally, comparative information of this kind should be of interest to students of primate classification. Animals providing specimens for this study were selected from over 800, representing a dozen or more species, located at the Delta Regional Primate Research Center, Covington, Louisiana. The classification employed is that outlined in the *Progress Report of the Primate Centers' Committee on Primate Nomenclature* (G. G. Simpson, Chairman). Semen specimens were collected from anaesthetized animals by electro-ejaculation (Roussel & Austin, 1968). They were incubated at room temperature for 30 min with an equal volume of 1% trypsin, in order to digest the coagulum; the coagulum appears in most ejaculates, and contains a concentration of spermatozoa about equal, by volume, to that of the liquefied plasma (Roussel & Austin, 1967a, b). Volume, sperm concentration, and the percentage of motile, eosin-negative, and morphologically abnormal cells were recorded. The specimens were sealed in 1·2-ml ampoules, and frozen in liquid nitrogen. All specimens were collected within a 60-day period beginning 14th September 1966. Human semen specimens were collected in Ann Arbor, and were treated and preserved in the same way. For subsequent studies (in Ann Arbor), the thawed cell suspensions were centrifuged at 800 *g*; the seminal plasma was taken off, and the packed cells were resuspended in 0·9% NaCl to the original volume of the specimen. The two fractions thus obtained, namely, the 'plasma fraction' and 'sperm fraction', were assayed for their content of lactic acid (Barker & Summerson, 1941), citric acid (Saffran & Denstedt, 1948) and fructose (Roe, 1934; Mann, 1948). The results are reported with reservation that the 'sperm fraction' must have been heavily contaminated by seminal plasma.

The characteristics of spermatozoa recorded in the trypsin-digested semen specimens before freezing are given in Table 1. The total concentrations of lactic acid, citric acid and fructose in the 'sperm-fraction' and 'plasma-fraction' are given in Table 2. These values are reported with the recognition that the variability found, between and within species, must have several sources, some of which may be artefacts of the methods of semen collection.

TABLE 1

CHARACTERISTICS OF SPERMATOZOA IN SEMEN OF PRIMATES (MEAN ± S.E.)

	No.	*Sperm concentration* (10^6 *sperm/ml*)	*Motile spermatozoa* (%)	*Eosin-negative cells* (%)	*Abnormal spermatozoa* (%)
Tupaia glis	1	90·0	50	69	8
Cebus apella	3	48·0 ± 15	28 ± 28	45 ± 12	41 ± 10
Saimiri sciureus	1	68·0	20	37	49
	3	286·0 ± 306	40 ± 14	56 ± 12	46 ± 15
	2	206·0 ± 48	30 ± 10	48 ± 8	51 ± 2
	2	54·5 ± 25	50 ± 0	60 ± 34	35 ± 6
Erythrocebus patas	1	74·0	30	61	40
	3	30·7 ± 21	30 ± 18	52 ± 20	35 ± 8
	7	445·9 ± 16	41 ± 9	66 ± 8	37 ± 8
	5	373·2 ± 17	56 ± 4	72 ± 7	25 ± 6
	3	6·5 ± 4	5 ± 0	19 ± 2	56 ± 1
Cercopithecus aethiops	4	288·0 ± 295	34 ± 7	45 ± 7	40 ± 6
	2	57·0 ± 7	38 ± 32	50 ± 39	26 ± 12
Macaca mulatta	3	442·6 ± 190	50 ± 7	60 ± 5	26 ± 10
	1	52·0	50	60	30
	8	31·7 ± 22	32 ± 2	57 ± 14	31 ± 14
	4	1288·5 ± 814	53 ± 0	74 ± 3	35 ± 11
	5	56·0 ± 44	33 ± 23	46 ± 25	28 ± 16
	1	110·0	50	64	32
	1	145·7	60	28	47
M. speciosa	1	214·0	30	57	50
	2	16·3 ± 4	5 ± 0	19 ± 0	33 ± 0
	2	509·0 ± 195	60 ± 0	77 ± 0	15 ± 0
	4	11·0 ± 5	28 ± 5	42 ± 11	29 ± 5
	1	307·8	55	79	10
M. irus	2	10·0 ± 4	35 ± 15	54 ± 10	36 ± 0
	5	164·8 ± 84	48 ± 16	66 ± 20	30 ± 8
Therada gelada	1	104·0	20	22	19
Hylobates lar	1	34·0	15	39	31
	1	176·0	5	20	58
	1	552·0	10	21	51
Pan troglodytes	4	749·5 ± 415	33 ± 15	50 ± 17	22 ± 9
	1	30·0	10	23	26
	3	852·3 ± 415	10 ± 7	26 ± 8	26 ± 4
	1	89·7	35	57	16
	1	346·5	20	48	57

On the assumption that the data reflect some specific biological variation, and that some of the parameters may be more important indicators of specific variability than others, two kinds of analysis were performed. Analysis of variance among all species for each variable was performed in order to find any significant differences between species for seminal characteristics. Two such

differences were found; it is, however, possible that further differences exist, which have been obscured by the small number of samples and by the large variability among individuals belonging to a given species. The tree shrew (*Tupaia glis*) differed from all other species in the very high citric acid content of the 'sperm-fraction' (mg/100 ml), F = 54·53 ($P<0·01$). The shrew and the gibbon (*Hylobates lar*) differed from the rhesus (*Macaca mulatta*) in having a much lower concentration (mg/100 ml) of fructose in the seminal plasma, F = 2·51 ($P<0·01$).

TABLE 2

CONCENTRATION OF LACTIC ACID, CITRIC ACID AND FRUCTOSE IN THE 'SPERM FRACTION' AND 'PLASMA FRACTION' SEPARATED FROM FROZEN–THAWED SPECIMENS OF SEMEN, AND CALCULATED IN MG/100 ML SEMEN

	No. of specimens	*Lactic acid*		*Citric acid*		*Fructose*	
		Sperm-fraction	*Plasma-fraction*	*Sperm-fraction*	*Plasma-fraction*	*Sperm-fraction*	*Plasma-fraction*
Tupaia glis	1	215·0	180·0	20·0	90·0	0	0
Cebus apella	3	0	4·0	13·3 ± 18·9	79·3 ± 85·3	0	563·3 ± 496
Saimiri sciureus	8	41·9	151·2	2·7 ± 4·3	48·0 ± 13·6	0·38 ± 0·99	110·4 ± 129
Erythrocebus patas	19	33·5 ± 21·3	194·0 ± 222	30·9 ± 52	126·7 ± 62	17·6 ± 39	314·6 ± 274
Cercopithecus aethiops	6	20·2 ± 12	192·3 ± 196	4·8 ± 9·5	121·6 ± 25·2	10·0 ± 15·5	264·0 ± 175
Macaca mulatta	23	31·6 ± 30	137·9 ± 115	1·9 ± 5·2	156·6 ± 86·1	14·0 ± 27·6	755·3 ± 904
M. irus	7	36·3 ± 16·7	238·7 ± 88·4	0	101·4 ± 65	7·1 ± 13·3	298·8 ± 264
M. speciosa	10	28·0 ± 30	183·4 ± 186	5·8 ± 9·1	231·1 ± 121	0	261·8 ± 108
Hylobates lar	3	—	—	—	—	0	3·3 ± 4·6
Pan troglodytes	10	24·0 ± 15·5	160·0 ± 127	14·9 ± 23·9	256·3 ± 191	10·4 ± 29·8	496·8 ± 363
Therada gelada	1	10·0	130·0	16·0	168·0	0	160·0
Homo sapiens	9	50·0 ± 29·1	95·6 ± 26·3	82·9 ± 112	347·1 ± 129	2·2 ± 6·3	55·6 ± 38

In order to identify the seminal parameters which contribute most significantly to the differences observed between animals and between species, factor analysis was performed. The varimax rotation of the principal axes solution of the correlation matrix accounts for all the variation by means of three uncorrelated factors. The first factor is clearly related to the fructose content of the specimens, especially that of the 'sperm-fraction'. The second relates to lactic acid and citric acid content of the 'sperm-fraction' expressed as mg/100 ml of cell suspension. The third factor is weakly or negatively related to all variables and accounts for the least total variability. It is either meaningless, or represents some semen characteristic which is inadequately measured by the indices employed here.

This work was supported by Grant No. 08950 NIH HD 02592-01.

REFERENCES

BARKER, S. B. & SUMMERSON, W. H. (1941) The colorimetric determination of lactic acid in biological material. *J. biol. Chem.* **138**, 535.

MANN, T. (1948) Fructose and fructolysis in semen in relation to fertility. *Lancet*, **i**, 446.

Roe, J. H. (1934) Colorimetric method for determination of fructose in blood and urine. *J. biol. Chem.* **107,** 15.

Roussel, J. D. & Austin, C. R. (1967a) Enzymic liquefaction of primate semen. *Int. J. Fert.* **12,** 288.

Roussel, J. D. & Austin, C. R. (1967b) Preservation of primate spermatozoa by freezing. *J. Reprod. Fert.* **13,** 333.

Roussel, J. D. & Austin, C. R. (1968) Improved electro-ejaculation of primates and some properties of the semen. *J. Inst. Anim. Tech.* (In press).

Saffran, M. & Denstedt, O. F. (1948) A rapid method for the determination of citric acid. *J. biol. Chem.* **175,** 849.