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Recommended Biochemical Parameters for Routine Semen Analysis

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Summary. The measurement of biochemical markers in human seminal plasma is important in the evaluation of male infertility. We recommend the measurement of one representative substance for each organ involved in seminal fluid production as a routine diagnostic tool: The initial fructose level for seminal vesicular function, citrate or acid phosphatase for the prostate gland and free carnitine as an index of epididymal function. A biochemical analysis of seminal fluid enables us to detect disturbances of the male adnexal organs and may lead to more exact therapy.

Key words: Seminal fluid, Biochemical analysis, Citrate, Fructose, Carnitine.

Introduction

A complete analysis of an ejaculate should include both a spermiocytogram (estimating the number, motility and morphology of spermatozoa) and a biochemical evaluation of the seminal fluid.

Mann [29] showed that both an adequate sperm count and normal composition of the seminal fluid are necessary for the ejaculate to fertilize an ovum.

An ejaculate without fructose or which lacks secretion from the seminal vesicles, is less effective. The prostatic secretion which contains enzymes such as acid phosphatase and acrosin, stimulates sperm motility and increases the fertilizing power of the fluid.

The normal ejaculate is a heterogeneous mixture of the secretions from three organs of the male genital tract. The greater part of the volume is secreted by the seminal vesicles about 50 to 65% with about 30 to 40% contributed by the prostate. Secretion from the testes, epididymes and vasa deferentia makes up about 3 to 5% of the total ejaculate (Fig. 1).

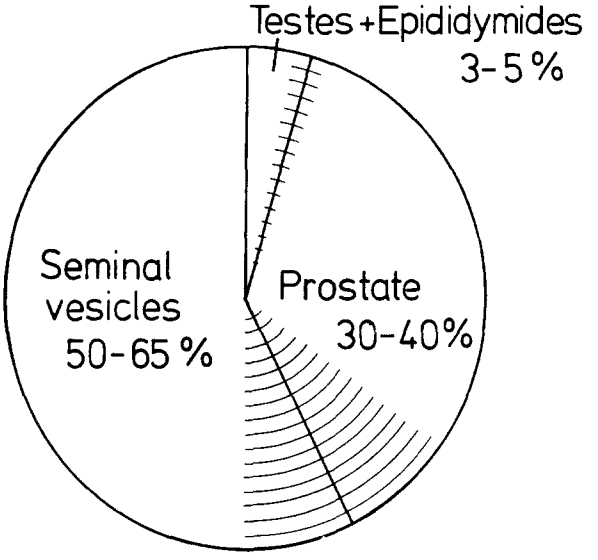

Fig. 1. Composition of seminal fluid

When the three components of the ejaculate are in normal proportions we speak of "eucrasia" of the seminal fluid. This, together a normal spermiogram offers the best indicator for satisfactory fertilisation. Efficient function of *all* the glands involved in seminal fluid production is essential for eucrasia to occur.

"Dyscrasia" of the seminal fluid occurs with a maldistribution of the secretions. This can often be recognized by an alteration in the pH, because of a change in the acidity of the prostatic secretion.

Material and Methods

Semen specimens were obtained by masturbation from patients consulting the infertility ambulance as well as healthy donors and men who had undergone vasoresection. Half an hour after ejaculation all biochemical estimations were made following deproteinization. Fructose was measured using a commercial kit for blood sugar

Table 1. The allocation of biochemical substances to their corresponding organs

Biochemical parameter \ Organ	Testis/ Epididymis	Seminal vesicles	Poststate
specific substances			
Initial fructose level	–	+	–
Initial citrate level	–	–	+
Acid phosphatase	–	–	+
Free carnitine	+	–	–
non-specific parameter			
Gamma-GT	+	–	+
Glutamic acid	+	–	+
Glycerophosphorylcholine	+	?	?

evaluation (Hexokinase method), converting fructose to glucose by phosphoglucose-isomerase as described earlier [42]. With the same deproteinizing procedure citrate was estimated enzymatically using the citrate lyase reaction [42].

Free carnitine was evaluated by an enzymatic-colorimetric method which has been modified by us by the application of an ordinary photometer [41]. Only the free carnitine is recovered in this method.

Prostatic acid phosphatase activity was measured both photometrically using p-nitrophenylphosphate as substrate [13] and, in a few specimens, also by radioimmunoassay. All enzymes were obtained from Boehringer Mannheim GmbH.

Results

Substances of particular diagnostic value are those which are produced in one particular region, and are not secreted from any other organ of the male genital system. Examples of such specific substances are fructose, prostatic acid phosphatase, citrate and free carnitine. The allocation of each substance investigated to its corresponding organ is shown in Table 1.

In contrast to these substances, others are found simultaneously in several different glands. The latter include gamma-GT [12, 19, 32, 33], glutamic acid [27] and Glycerophosphorylcholine [2, 6].

1. Fructose

Fructose has been taken as an index of seminal vesicle secretion [28]. It was very soon used in routine diagnostic tests. Much work has underlined the importance of fructose for the assessment of a spermiogram (Table 2). Fructose is metabolized by the spermatozoa producing energy release. This break-down of fructose – fructolysis – correlates with the number of motile sperms [39]. In order to obtain values that may be used for comparison, it is necessary to determine the initial fructose level, i.e. the concentration reached 30 min after ejaculation.

The epithelium of the seminal vesicles requires androgens in order to form fructose from glucose and to concentrate

Table 2. Biochemical parameters for routine semen analysis

Biochemical parameters	Corresponding organ	Normal range	References
Fructose	Seminal vesicles	> 150 mg%	Mann (1946, 1974) Harvey (1951) Schirren (1960, 1963, 1971) Lischka (1975) Schill (1976)
Citrate	Prostate	250–800 mg%	Scherstén (1930) Huggins and Neal (1943) Humphrey and Mann (1948, 1949) Lundquist (1949) Hensel and Hornstein (1970, 1971) Wetterauer and Heite (1976)
Acid phosphatase	Prostate	200–800 U/ml	Kutscher and Wolbergs (1935) Gutman and Gutman (1938) Lundquist (1946, 1947) Kimmig et al. (1967) Schirren (1971) Heite and Wetterauer (1979)
Free carnitine	Epididymis	> 4 mg%	Marquis and Fritz (1965) Casillas (1972, 1973) Brooks et al. (1974) Frenkel et al. (1974) Lewin et al. (1976) Wetterauer and Heite (1976, 1978, 1980)

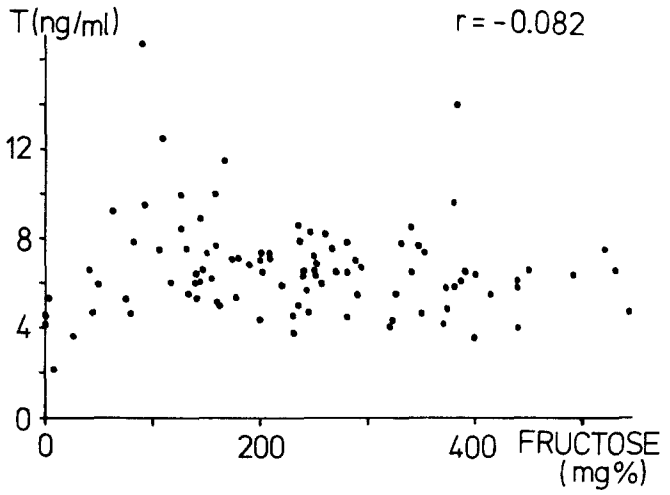

Fig. 2. Fructose is plotted against plasma testosterone in 98 patients: no substantial correlation detectable

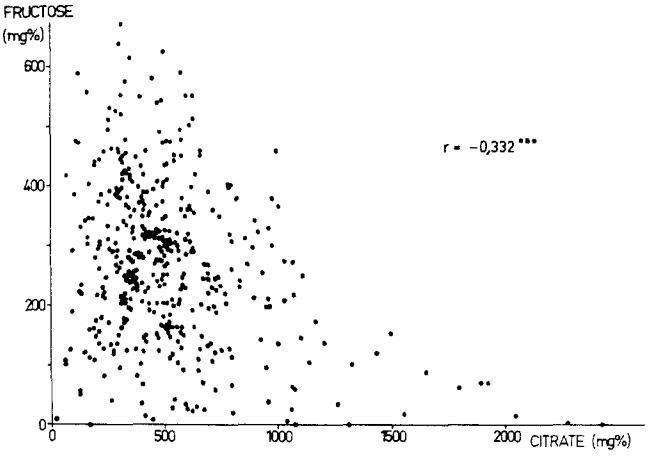

Fig. 4. Fructose is plotted against citrate levels in 400 unselected semen specimens (coefficient of correlation $r = -0.332; p < 0.001$)

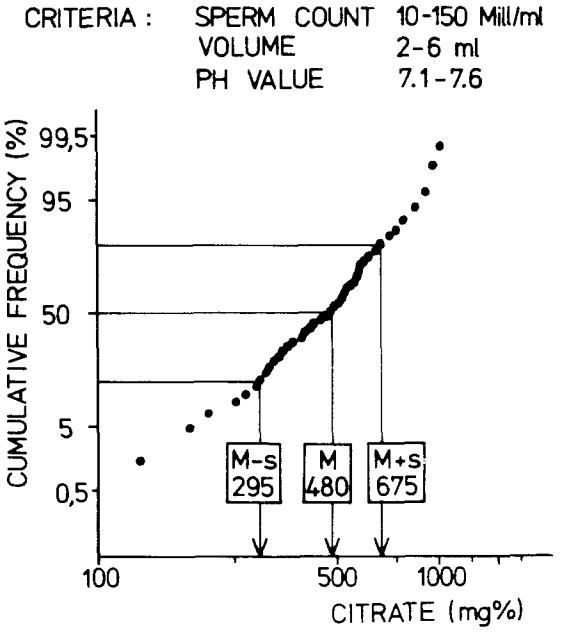

Fig. 3. Frequency distribution of citrate levels in 125 selected semen specimens (every third value is marked)

it to a level well above that of the blood sugar. However, there is no direct correlation between the concentration of fructose and the blood testosterone level (Fig. 2).

A low fructose content in the seminal fluid can be due to different causes. It can be due to

- an overall deficiency of male sex hormones, which prevents the seminal vesicles from producing sufficient fructose,
- scarring of the seminal vesicles resulting in such a degree of atrophy that adequate fructose production is no longer possible,
- blockage of the ejaculatory ducts or of the colliculus seminalis.

One should not assume that depletion of the initial fructose level is associated with a post-pubertal Leydig cell deficiency, nor that a hormonal dysfunction is necessarily present. However, measurement of the fructose concentration cannot be used as a substitute for the direct determination of hormone levels.

2. *Citrate* (Table 2)

As early as 1930 Scherstén [34] detected citrate as a component of the seminal fluid. It is certainly not reasonable to limit diagnosis solely to fructose determination, when a substantial amount of the seminal fluid is derived from other glands. Citrate is produced almost exclusively in the prostate, and its concentration in the secretion of this organ amounts to over 2,500 mg% [5, 9]. In the seminal vesicles, on the other hand, only a trace is found [16].

The normal range of citrate concentration lies between 250 and 800 mg%. This figure was derived from citrate determinations in 125 euspermic patients (see the frequency distribution shown in Fig. 3).

Extremely high citrate levels (over 2,000 mg%) occur when there is a complete failure of the secretion of the seminal vesicles; when, for instance, the colliculus seminalis is blocked [43]. Lowered citrate values are associated with functional disturbances of the prostate, such as prostatitis [36, 38] or malignant change [21].

The significant negative correlation between citrate and fructose levels (Fig. 4) can be explained by the inverse relationship between the secretions of the prostate and seminal vesicles. Should, for example, the secretion of the seminal vesicles predominate, high fructose and relatively low citrate values will be found in their combined fluid output [11, 40].

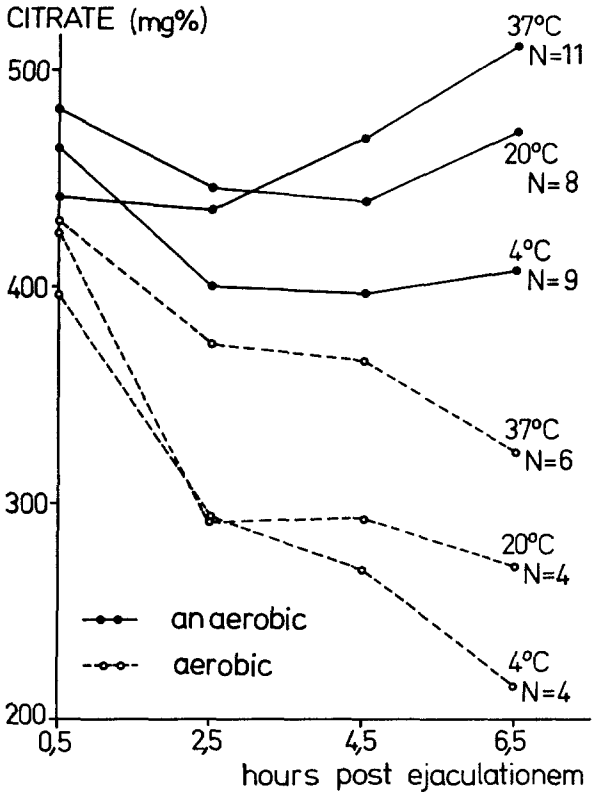

Fig. 5. Variation in citrate concentration during storage of ejaculates at different temperatures under aerobic and anaerobic conditions (so-called citricolysis)

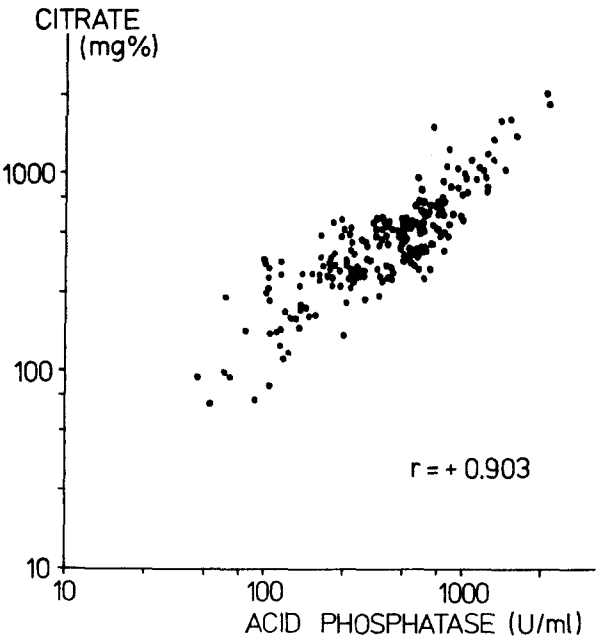

Fig. 6. Citrate is plotted against prostatic acid phosphatase activity in 200 semen specimens (coefficient of correlation $r = 0.903$; $p < 0.001$)

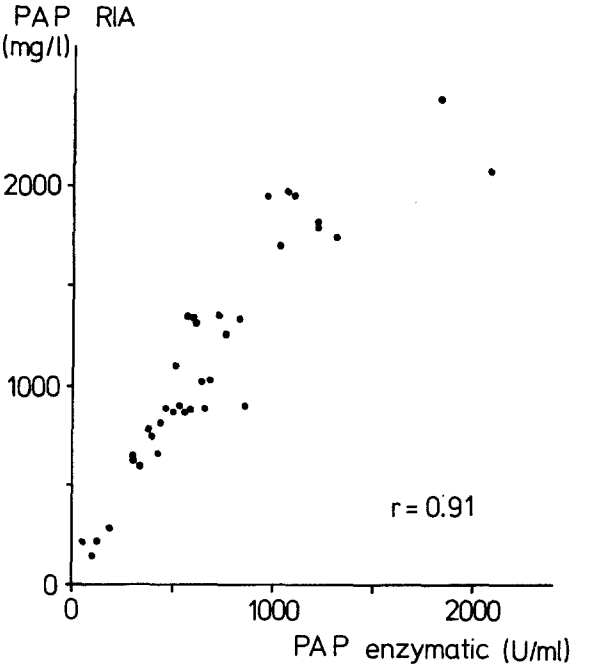

Fig. 7. Prostatic acid phosphatase measured with RIA is plotted against colorimetrically determined PAP ($r = 0.91$)

As is also the case with fructose, the citric acid concentration undergoes a change when ejaculate is stored. This so-called "citricolysis" is dependent on temperature and requires the presence of motile spermatozoa. Figure 5 shows the change in the citrate concentration during the storage of the ejaculate at various temperatures. A clear decrease takes place under aerobic conditions, and at 20 °C the level falls by 33% within 2 h. The *increase* of citrate concentration during anaerobic storage at 37 °C is remarkable, the conditions being similar to those prevailing in the posterior fornix [1, 41].

This is of practical significance, since only by determining the "initial citrate value" (30 min after ejaculation) can figures be obtained which are suitable for making comparisons.

3. Prostatic Acid Phosphatase (Table 2)

The introduction of acid phosphatase as an aid to the diagnosis of prostatic cacinoma [10] has excited a great deal of interest. Its concentration in the seminal fluid is about 50,000 times higher than in the blood. As with citrate, acid phosphatase is a specific indicator of prostatic function. Its level shows such a close correlation with the citrate concentration ($r = 0.903$, see Fig. 6) that it is sufficient for routine diagnosis to estimate only one of these substances.

It appears that measurement of the prostatic acid phosphatase in the seminal fluid with radio-immuno assay (RIA) gives the same information as does the above mentioned colorimetric measurement. Figure 7 which illustrates the high correlation of the values of both substances, confirms that either may be used diagnostically.

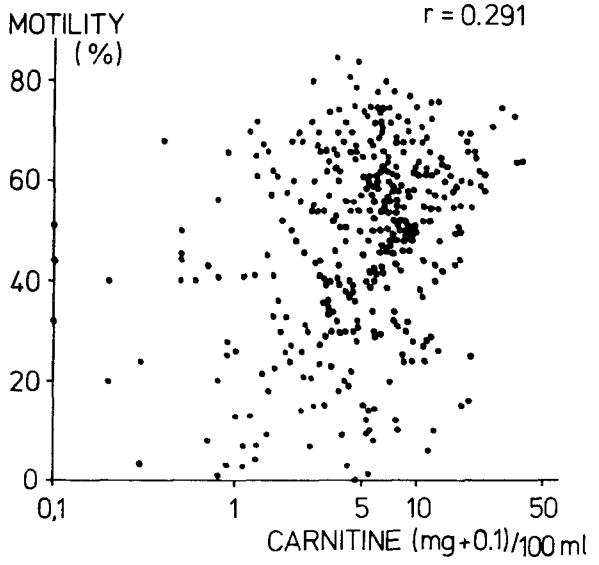

Fig. 8. Carnitine is plotted against sperm motility in 360 unselected semen specimens (coefficient of correlation $r = 0.291$; $p < 0.001$)

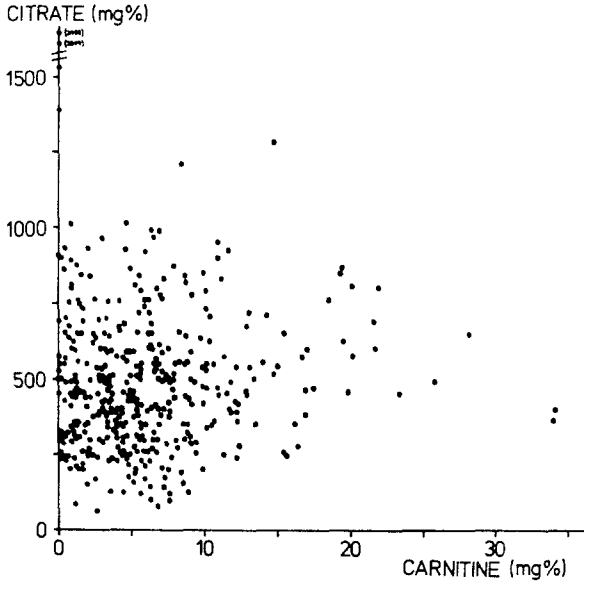

Fig. 9. Citrate is plotted against carnitine in 400 semen specimens: no substantial correlation detectable

For routine research, however, we prefer to use the citrate estimation, since this is the simpler and cheaper method and because this estimation can be made at any time, even if the protein element of the specimen has been denatured before storage [42].

4. Free Carnitine

Carnitine (Table 2) has been shown in recent years to be an indicator of epididymal secretion, following the work of Casillas. According to our own investigations, 95% of free carnitine is produced by the epididymis.

Table 3. Comparison of carnitine values in euspermic patients with those of patients subjected to vasectomy

	Median value	16-per-centile	84-per-centile
100 euspermias	7.0	4.3	10.5
80 vasoresections	0.4	0.1	0.8

Carnitine plays an important part as a "carrier" in the transport of fatty acids through membranes [8]. It is not present in the testicular spermatozoa, and its concentration continuously increases as they pass to the cauda epididymis. It is assumed that a correlation exists between the absorption of carnitine by the spermatozoa during their passage through the edididymis and the development of their fertilizing capability [4].

Carnitine showed a remarkably high correlation with the sperm count ($r = 0.603$) in 400 unselected andrological patients. A positive correlation with sperm motility ($r = 0.291$) was detected (Fig. 8).

In contrast to fructose and citrate, free carnitine is the only substance which showed positive correlations with both sperm count and motility. It therefore may give an indication of the fertilizing capability of an ejaculate, and it may even correlate with fertility itself.

Patients with an idiopathic azoospermia have markedly lowered carnitine values (median value = 0.9 mg% in 60 patients), as do those with severe oligozoospermia (median value = 1.7 mg% in 50 patients). As against this, 100 men with euspermia showed a median value of 7.0 mg%; which was significantly different from the previous groups mentioned.

In order to demonstrate that the free carnitine level is a specific indicator of epididymal function, one has to exclude any possible influence of the secretions from the seminal vesicles and prostate.

No kind of correlation occurred between carnitine and either citrate (Fig. 9) or fructose [44].

If one compares the median and scatter for the carnitine values in men with euspermia with the values taken from patients subjected to vasectomy (Table 3), it is apparent that the testicular-epididymal secretion determines almost exclusively the carnitine content of the ejaculate.

Discussion

It is not reasonable to limit biochemical semen analysis to fructose estimation as an index of seminal vesicular function, when a substantial amount of the seminal fluid is also derived from other glands of the male genital system. Isolated disturbances of the function of the seminal vesicles are rare. It seems to be of diagnostic significance, to reveal disturbances of prostatic and epididymal function by means of specific biochemical parameters.

A detailed biochemical analysis reveals specific patterns for seminal duct obstructions and post-inflammatory conditions of the male adnexal organs. In infertility a biochemical semen analysis together with a morphological evaluation of the spermatozoa – the latter still is undoubtly the basal component of a spermiogram –, contributes to a precise statement about the extent and localization of the underlying disturbance; a prerequisite for optimal therapy.

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