CITRIC ACID IN HUMAN PROSTATIC SECRETION AND METASTASIZING CANCER OF PROSTATE GLAND

BY

H. MARBERGER, M.D.**EVE MARBERGER, M.D.***Departments of Surgery and Experimental Pathology,
University of Innsbruck***T. MANN, M.D., Ph.D., Sc.D., F.R.S.**

AND

CECILIA LUTWAK-MANN, M.D., Ph.D.*A.R.C. Unit of Reproductive Physiology and Biochemistry,
University of Cambridge*

Citric Acid in Human Semen as Indicator of Prostatic Function

Human semen, like that of certain large domestic animals, contains citric acid in a concentration varying from 100 to 1,000 mg./100 ml. Among other characteristic chemical constituents of human semen is fructose, which occurs in amounts varying from 40 to 400 mg./100 ml. Whereas, however, in some animals, such as the bull for example, citric acid and fructose are both formed in the same accessory organ of reproduction—namely, the seminal vesicle—in man citric acid is produced by the prostate gland, while fructose is secreted mainly by the seminal vesicles, and to a small extent also by the ampullae. Therefore determinations of citric acid and fructose in ejaculated semen, which can be done by simple and accurate methods (Mann, 1951, 1954), provide a means whereby to assess quantitatively the secretory function of the prostate and seminal vesicle, respectively, in the living individual, under normal as well as pathological conditions (Humphrey and Mann, 1949; Lundquist, 1949; Mann and Lutwak-Mann, 1951; Harvey, 1951, 1957; Schirren, 1961).

In cases of occlusion of the ejaculatory ducts through which normally the seminal vesicles and ampullae void jointly their contents into the urethral canal, the semen is not only azoospermic but, moreover, lacks fructose (Young, 1949). Recently we have examined the semen of a 31-year-old man who was sterile but had normal libido and a normal ejaculatory mechanism, as well as normally functioning testes (confirmed by testicular biopsy). This patient consistently produced ejaculates which, although only slightly subnormal in volume, had a pH value of 6.6 and contained neither spermatozoa nor fructose. On analysing such an ejaculate for citric acid, we found a content of 2,145 mg./100 ml. This is greatly in excess of normal values, and is characteristic of pure prostatic secretion rather than a proper complete ejaculate. Surgical exploration in this case revealed a congenital absence of both vasa deferentia and of the ejaculatory ducts.

In contrast to the above-described case of male sterility, which was characterized by absence of fructose, absence of spermatozoa, but presence of citric acid in semen, we have encountered another interesting abnormality in a 48-year-old man of proved sterility whose ejaculates, when examined on several occasions in the

course of the last three years, contained only a negligible amount of fructose—namely, 1.7–13 mg./100 ml.—but were at the same time distinguished by an extraordinarily high sperm density. The most recently examined ejaculate of this man had a volume of 1.7 ml. and a fructose content of 6.3 mg./100 ml.; at the same time sperm density was 600 million cells/ml., about 50% of the spermatozoa being motile; the pH of the semen was 7.3. The citric acid content of this ejaculate was 1,050 mg./100 ml., a high value which, however, could still be regarded as falling within the range of normal variations. As no surgical exploration was performed, the anatomical basis and aetiology of this condition remain obscure, but several possibilities come to mind—namely, that this individual lacks functional seminal vesicles, and consequently is incapable of producing seminal fructose; that the seminal vesicles, though present, are unable to void their secretion into the urethra because of an occlusion; that they generate fructose, but the latter is metabolized before instead of after sperm ejaculation; and that, as well as being deprived of fructose (as their substrate), the spermatozoa are abnormal in some other fundamental respect, possibly with respect to nucleoprotein, and consequently incapable of fulfilling the normal fertilizing function.

Citric Acid in Metastases of Prostatic Cancer

It has previously been shown (Lutwak-Mann, Mann, and Price, 1949) that when a minute fragment of a citric-acid-secreting accessory organ of a male rat is dissected and transplanted under the skin of a castrated male or an intact female rat, such a subcutaneous graft will grow and produce citric acid under the influence of injected testosterone. There is also ample evidence from studies on man that the growth of prostatic cancer, as well as its metastases, is stimulated by androgenic steroids (Huggins, 1956). So far, however, it has not been demonstrated whether androgen-controlled metastases of prostatic cancer share with the primary tumour the ability to produce citric acid. In an attempt to explore this possibility we have analysed and compared the content of citric acid in cancerous prostatic tissue itself as well as in the metastases of that cancer in other organs of the body. The conclusion which we have reached is that citric acid is definitely produced in the metastatic tissue, but its concentration, like that of acid phosphatase (Marberger, Riedesel, Anderson, and Malek, 1956), is lower in the metastases than in the primary tumour, and much below that which one usually encounters in the non-cancerous prostate gland.

In the case of a primary untreated adenocarcinoma of the prostate gland in a 65-year-old man we found 113 mg. of citric acid/100 g. in the tumour itself, and 46 mg. of citric acid/100 g. in the enlarged para-aortal lymph nodes, which consisted largely of solid undifferentiated cancerous tissue and only to a smaller extent of typical adenoid formations.

Another case was that of a 48-year-old man who had been orchidectomized two years previously, when the prostatic cancer was first diagnosed, and was subsequently treated extensively with oestrogens and finally adrenalectomized. When death supervened four weeks after adrenalectomy we dissected from the liver pathologically unchanged tissue separately from the cancer metastases. The normal liver tissue contained 2.3 mg. of citric acid/100 g., but in the liver metastases

we found 28.4 mg./100 g. The para-aortal and inguinal lymph nodes contained 6.9 and 4.5 mg. of citric acid/100 g. respectively, but in the lymph nodes (para-aortal and peribronchial) obtained from two patients who underwent surgery for non-prostatic diseases the citric acid content was only 0.8 and 1.6 mg./100 g., respectively.

The third case was that of a 62-year-old man with an adenocarcinoma of the prostate and a polymorph-cell cancer of the bladder; for three years before his death this man had been treated with oestrogens. A sample of tissue from the prostatic tumour itself contained 30.8 mg. of citric acid/100 g. The iliacal lymph nodes, which appeared to be invaded largely by the cancer of the bladder, and to a smaller extent by that of the prostate, contained 6.5 mg. of citric acid/100 g.

A fourth case was that of a 63-year-old man with a recently diagnosed adenocarcinoma of the prostate. This patient had been treated with oestrogen during a period of five days before the resection of the tumour, and orchidectomized only two days prior to that. For analysis of citric acid, samples of tissue were removed during the transurethral resection of the prostate, from two distinct regions of the prostatic cancer—namely, one close to the urethra, and the other from the region near the capsule; these two samples were found to contain, respectively, 34 and 28 mg. of citric acid/100 g.

Citric Acid in the "Bladder-irrigation Fluid" and Blood During Prostatectomy

In the course of the transurethral resection of the prostate performed on the last-mentioned patient additional analyses of citric acid were made on the fluid (water) used for irrigation of the bladder, and on blood samples taken immediately prior to surgery and subsequently at half an hour, one hour, and 24 hours. We attempted to find out, by analysing the irrigation fluid, how much citric acid is washed out from the prostatic tissue and secretion in the course of the operation. The blood analyses were done in order to examine the possibility whether the surgical procedure and the manipulations involved therein could cause the entry of citric acid from the prostate into the blood-stream. In this connexion an earlier observation may be recalled that prostatic massage or surgery can lead to a diffusion of acid phosphatase from the prostate gland into the blood, as a result of which there occurs a significant but transient rise in the level of acid phosphatase in blood serum (Marberger, Segal, and Flocks, 1957). The citric-acid values in blood serum which we observed were (mg./100 ml.): 2.0 at the beginning of the operation, 3.2 at half an hour, 3.6 at one hour, and 1.95 after 24 hours. A control sample of blood obtained from a normal man gave a value of 2 mg. of citric acid/100 ml. of serum. The irrigation fluid (10 litres) was concentrated by evaporation in the presence of sulphuric acid to about one-twentieth of its original volume; a total of 11.7 mg. of citric acid was recovered, which probably represented a large proportion of citric acid initially present in the prostate gland.

An analogous series of analyses involving determinations of citric acid in the prostate tumour, blood, and bladder-irrigation fluid was carried out on material obtained from a 76-year-old man in whom prostatectomy was undertaken because of a growth diagnosed as non-malignant glandular hyperplasia of the prostate.

The citric acid content of the hypertrophied prostate was 107.4 mg./100 g. in tissue removed from the gland near the capsule, and 58.4 mg./100 g. in the urethral portion. The blood-serum values (mg. citric acid/100 ml.) were 2.20, 2.75, 3.40, and 1.63 in samples withdrawn respectively at the beginning of the operation and after half an hour, one hour, and 24 hours. A concentrate from the irrigation fluid contained 32.4 mg. of citric acid, thus even exceeding the high content of citric acid encountered in the previous case.

Summary

Chemical determinations of citric acid in human semen provide a simple and convenient means for a quantitative assessment of the secretory function of the prostate gland. A case is described in which, owing to congenital absence of the seminal and ejaculatory ducts, the ejaculated semen consisted almost entirely of prostatic secretion, as indicated by an abnormally high level of citric acid and complete absence of spermatozoa and of fructose.

The cancerous prostate shares with the normal gland the ability to secrete citric acid, and the metastases of prostatic adenocarcinoma also produce citric acid as a characteristic chemical constituent, but at a distinctly lower level.

In the course of transurethral resections of the prostate gland we found that, as a result of the surgical procedure, a relatively large amount of citric acid of prostatic origin passes into the fluid used for bladder irrigation. There was also an indication that the manipulations involved in prostatectomy caused a slight and transient rise in the citric-acid level of the peripheral blood.

We are much indebted to Dr. Födisch, of the Pathological Institute, University of Innsbruck, for the histopathological examination of the organs, which represented a material contribution to this study. The support of the Population Council is gratefully acknowledged. We also thank Miss Herta Ender for her skilful technical assistance.

REFERENCES

- Harvey, C. (1951). *Proc. Soc. Study Fertil.*, **3**, 56.
 — (1957). *Ibid.*, **8**, 3.
 Huggins, C. (1956). *Cancer Res.*, **16**, 825.
 Humphrey, G. F., and Mann, T. (1949). *Biochem. J.*, **44**, 97.
 Lundquist, F. (1949). *Acta physiol. Scand.*, **19**, Suppl. 66.
 Lutwak-Mann, C., Mann, T., and Price, D. (1949). *Proc. roy. Soc. B*, **136**, 461.
 Mann, T. (1951). *Proc. Soc. Study Fertil.*, **3**, 50.
 — (1954). *The Biochemistry of Semen*. Methuen, London.
 — and Lutwak-Mann, C. (1951). *Physiol. Rev.*, **31**, 27.
 Marberger, H., Riedesel, R. D., Anderson, D. O., and Malek, L. H. (1956). *J. Urol.*, **75**, 857.
 — Segal, S. J., and Flocks, R. H. (1957). *Ibid.*, **78**, 287.
 Schirren, C. (1961). *Fertilitätsstörungen des Mannes: Diagnostik, Biochemie des Spermaplasmas, Hormontherapie*. Beitrage zur Sexualforschung, Heft 22. Enke Verlag, Stuttgart.
 Young, D. (1949). *Brit. J. Surg.*, **36**, 417.

In Tasmania on February 8 the annual conference of Police Commissioners of the South Pacific area heard a report on the use of breath analysis and blood tests in the detection of drunken drivers. The report was given by the Chief Commissioner of Police in Victoria, Major-General Porter. He said that police officers and members of the Victorian Government believed that the "breathalyser" machine had already shown its value as a deterrent to drunken driving. Victoria so far is the only Australian State to have adopted compulsory breath analysis tests. (*Radio Australia News*, February 9.)