tions in values obtained at admission (correlation coefficient 0.807, $p < 0.001$). There was no significant correlation between the infarct size, as measured by the peak level of aspartate aminotransferase, and the peak values of T_4, FT_4 fraction or FFA.

Discussion. The increased FT_4 fraction in our study reflects a weaker protein binding of the hormone. A number of factors have been suggested that may affect this protein binding, and some of them may be active in MI; e.g. decreased thyroxine binding prealbumin levels caused by the 'acute phase reaction'[8,9], increased secretion of catecholamines and cortisol[10,11], metabolic acidosis[12], elevated body temperature[13], and increased serum FFA. It has been suggested by Hollander et al.[14] that FFA act as competitive inhibitors of T_4 binding. Our results, with significant correlation between FFA and FT_4 fractions on admission, support this hypothesis. However, increased FFA cannot fully explain the increased FT_4 fraction, since the former normalizes so quickly. Other factors mentioned above may therefore contribute.

Does an increase in free T_4 in serum imply a correspondingly increased hormone effect in tissues? T_3 is deiodinated to 3,3′,5-triiodothyronine (T_3) or 3,3′,5′-triiodothyronine (reverse T_3). Reverse T_3 is metabolically inactive. Other investigators have shown that in MI there is a reduced peripheral conversion of T_4 into T_3 with a concurrent increase in reverse T_3 production[8,14]. The mechanism of this shift in T_4 metabolism is unknown, but it certainly leads to lower concentrations of T_3 together with the increased FT_4 fraction. Thus, the level of hormone activity is not easily discerned by looking directly at concentrations. Assumptions can, however, be made indirectly by looking for T_4 effects in MI. Theoretically, high thyroid activity could be expected to shorten the S-T segments in patients with MI, as action potentials in hyperthyroid animals are shortened[16]. We could not confirm this on the electrocardiograms in our patients. The increase in thyroid hormones may be too moderate to induce electrocardiographic changes.

The fatty acid composition of serum is, however, susceptible to the T_4 concentration, and the composition in MI is characteristic for high thyroid activity[3,4]. We therefore believe that hormone effects are increased in MI, probably because of the increase in FT_4 shown in this study. In heart muscle this increase could lead to greater oxygen consumption, and the myocardium might suffer further damage. T_4 also sensitizes the myocardium to catecholamines and might induce arrythmias. A high FFA level has been shown to be arrythmogenic[17]. Patients with the combination of increased FFA and increased FT_4 fraction could thus be prone to develop arrythmias. However, our sample is too small to assess the relation between FFA, the FT_4 fraction, and the clinical course of MI.

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Testosterone in royal jelly[1]

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Summary. A compound with immunoreactivity, and TLC and GLC mobility of testosterone was isolated from royal jelly of honeybee *(Apis mellifera)*. This is the first demonstration of vertebrate steroid hormone in this species.

The hormone-like effects of royal jelly (larval food of honeybee *Apis mellifera*) in insects[2,3], experimental animals[4,5], and humans[6,7] have evoked considerable interest among scientists. The results of immunological and biological assays suggest that this material exhibits insulin- and gonadotropin-like activities[8-11]. The corticoid activity of royal jelly has been demonstrated in its acetone extracts, while its chloroform extracts contain estrogenic- and androgenic-like activities[13,14]. However, to date except for cholesterol[15,16], no other vertebrate steroid was isolated from this material. In this report, we present further evidence for the presence of testosterone in royal jelly.

Material and methods. Content of testosterone was determined in:

1. Native royal jelly (RJ) samples from Sigma Chemical Co. (Sigma 1981) and from OAC-Apiary (Guelph, Canada), collected in 1956 (OAC-1956) and in 1980 (QC-1980).
2. Lyophilized RJ samples from OAC-Apiary, collected in 1945 by G.F. Townsend (GFT-1945) and in 1981 by R.W. Shuel (RWS-1981), and in Prairie Vien Honey Co. (Michigan, USA) collected in 1981 and lyophilized in Dr Shuel's laboratory. All samples (except Prairie-1981 and Sigma-1981) were stored at −15 °C until processed. The samples from Prairie Honey Co. and Sigma Chemical Co.,

were exposed to higher temperatures (15-20 °C) during shipment and handling. All samples of RJ used in this work, except for that purchased from Sigma Chem. Co., were obtained through courtesy of R.W. Shuel, Ontario Agricultural College, Department of Environmental Biology, University of Guelph, Ontario, Canada.

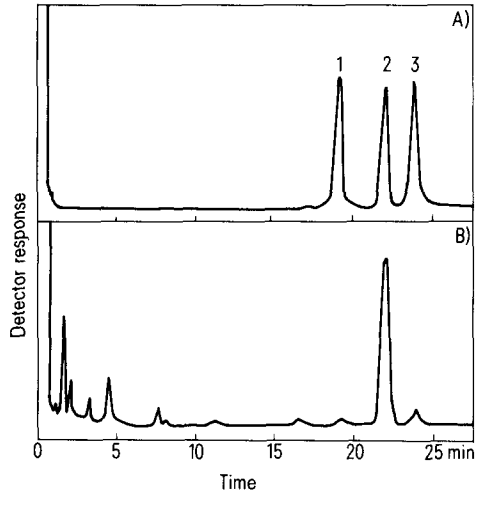
Determination of testosterone in RJ by radioimmunoassay (RIA). Samples of RJ (200 mg native form and/or 60 mg of lyophilized) were suspended in 2 ml of distilled water or Tris-HCl buffer (pH 7.4). 5 aliquots (200 µl) from each sample were extracted with ether (10 ml) and testosterone was determined in the dry residue (after evaporation of ether) by RIA method using commercially available test kit produced by CIS International (Sorin Biomedica, Italy). The lowest detectable dose of the method is 2.3 ± 1.2 pg of testosterone per assay tube, with a coefficient of variation of 6% within and 9% between the assays. Some RIA assays were also performed with antiserum from Wien Laboratories (Succasunna, NJ).

Isolation and identification of testosterone. 50 g of RJ (Sigma Chemical Co.) suspended in 20 ml of distilled water was extracted with 125 ml of methanol/chloroform (4:1, v/v). This extract was then concentrated in rotary evaporator and re-extracted with 50 ml of ether. After evaporation of ether (under N_2), the residue (2 ml of yellow oily substance) was dissolved in 50 ml of 0.5 N NaOH solution and re-extracted with 50 ml of ether. Dry residue from ether extraction was dissolved in 10 ml of 90% methanol and re-extracted with 50 ml of petroleum ether (boiling point 37-57 °C). Petroleum ether extract was discarded. The methanol solution was concentrated in vacuo, and the steroid re-extracted twice with 20 ml of ether.

Content of testosterone in royal jelly

Sample	Testosterone (ng/g)
Native royal jelly: OAC-1956	11.63 ± 0.4*
QC-1980	12.16 ± 0.2
Sigma-1981	11.12 ± 1.7
Lyophilized royal jelly: GFT-1945	36.16 ± 2.6
RWS-1981	36.34 ± 4.5
Prairie-1981	31.86 ± 1.3

* Mean ± SD; N = 5.



Gas liquid chromatogram of the trimethylsilyl derivatives of steroid standards (A), and the testosterone isolated from royal jelly (B). 1, 5α-Dihydrotestosterone; 2, testosterone; and 3, estradiol.

Dry residue from ether extract was dissolved in methanol and chromatographed on thin-layer plates in the presence of authentic testosterone (17β-hydroxyandrostene-3-one, purchased from Steraloids), using chloroform/methanol (98:2, v/v) as developing solvent system. Ascending thin-layer chromatography (TLC) was performed on 5 × 20 cm glass plates coated with silica gel 60 (250 µm thickness). Fraction corresponding to testosterone was then scraped into sintered glass filter funnel and the gel was eluted with 2 ml of methanol. A portion of the extract was dried, derivatized with the silylating reagent[17], and analyzed by gas-liquid chromatography (GLC) for testosterone. GLC was performed with a Beckman GC-65 instrument equipped with glass column (180 × 0.2 cm) packed with 3% SE-30 on Gas Chrom-Q (80 × 100 mesh). Temperature was programmed at 1.5 °C/min from 170 °C to 240 °C.

Results. Results obtained by RIA method demonstrated that RJ contains testosterone-like immunoreactive compound. Of the 2 antisera tested, the Wien serum which has greater cross-reactivity with other steroids than Sorin serum gave consistently lower values for testosterone (i.e., Sigma-1981 RJ showed 10.92 ± 1.50 ng/g with Wien antiserum and 11.22 ± 1.70 ng/g with Sorin antiserum). Therefore further assays were carried out with Sorin antiserum. As shown in the table, there was no significant variations in the content of this steroid between the samples of RJ from various locations. In addition, the content of the steroid found in the old samples was similar to that in the fresh samples.

The sequential extraction of RJ with methanol/chloroform and ether yielded a white powdered residue. This residue dissolved in methanol, was chromatographed on TLC and the band corresponding in migration to the authentic testosterone was isolated and subjected further to identification by GLC. As shown in the figure the isolated steroid had retention index identical to that of testosterone standard. Thus, the results of RIA, TLC and GLC suggest that the steroid isolated from royal jelly is testosterone.

Discussion. The insects, like vertebrates, possess Δ^{22}- and Δ^{24}-sterol reductase enzyme systems converting phytosterols to cholesterol[18]. Cholesterol serves as a precursor for the biosynthesis of biologically active metabolites. An inhibition of these enzyme systems in various insects causes a disruption of metamorphosis and larval development[19]. It is of interest that in addition to 20-hydroxy ecdysone (molting hormone), a known cholesterol metabolite in insects, other steroids, such as testosterone and 11-corticosterone (known cholesterol metabolites in vertebrates), were found in water beetles[20,21]. These hormones are produced by the prothoracic gland and stored in the pygidial bladders from which they are ejected to protect these insects from microorganisms and water dwelling predators[20,21].

Royal jelly is the product of salivary glands (hypopharyngeal and mandibular) and the honey sac of the nurse bee[22,23]. The significance of the presence of testosterone in this larval food of honeybee *(Apis mellifera)* is not yet known. In humans, free steroid hormones in the circulation freely penetrate through the glandular barrier and concentrate in saliva[23]. It is possible that testosterone in royal jelly has a similar hemolymph origin.

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Calcium transients in a crustacean motoneuron soma: Detection with arsenazo III

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Summary. Long lasting action potentials can be triggered in crayfish giant motor neurons by a short depolarizing pulse under different conditions. The concomitant increase in absorbance of the Ca indicator arsenazo III preloaded into the soma, confirms previous observations suggesting that these potential changes are related to a Ca inward current through the soma membrane.

The giant motoneurone (MoG) is a large cell, found bilaterally in the crayfish abdominal ganglia. Early work[1] suggested that the cell soma does not show any regenerative action potential in response to a depolarizing pulse, but it is now known that regenerative responses can be recorded under certain conditions. For instance, when the K^+ conductance of the membrane is reduced (either by TEA[8] or following inactivation by a conditioning depolarisation), a slow action potential (SAP) may be triggered by a short depolarization. This response is most probably due to an influx of Ca^{2+} ions, since it depends upon the presence of Ca^{2+}, but not Na^+ in the bathing solution, and is blocked by Co^{2+} and Mn^{2+}, but not by TTX[2]. In the preparations pretreated with TEA, the SAPs can also be recorded at more hyperpolarized levels than the normal resting potential. TEA is effective either injected into the MoG soma or added to the saline[3]. In this paper most of the results were obtained in this way when TEA was added to the solution.

We report below that the internal free Ca concentration increases during the SAP, as demonstrated by the change in absorbance of the Ca^{2+} indicator dye arsenazo III preloaded into the MoG soma. This observation strengthens previous observations suggesting that SAP is associated with an influx of Ca^{2+} ions.

Methods. Experiments were carried out on the giant motor neurons of the crayfish *Procambarus clarkii*. The ventral chord was dissected and perfused with Van Harreveld's solution containing (in mM): NaCl 195; KCl 5.4; $CaCl_2$ 13; $MgCl_2$ 2.6; Tris maleate 10, adjusted to pH 7.2 by NaOH. The 2nd, 3rd and 4th ganglia were desheathed and used for the experiments. The membrane potential was monitored by conventional electrophysiological techniques using a 3 M KCl electrode. A 2nd electrode filled with an aqueous solution of 1 mM arsenazo III (Sigma grade 1) was also inserted in the MoG soma. The dye was injected by negative current pulses (500 msec, 1 Hz) which hyperpolarized the cell by 30–40 mV. The injection was stopped when the soma appeared just detectably stained as judged by eye. The Arsenazo electrode was then used for passing both the conditioning depolarizing current pulse and the brief (2–5 msec) pulse triggering the SAP. The intracellular calcium transients were monitored by recording the differential absorbance of the dye at 650 and 700 nm as previously described[4,5].

Results. Typical responses obtained in TEA-treated preparations are shown in figure 1. The SAP was almost unchanged in Na-free solutions (fig. 1A). On the contrary, when Ca^{++} was omitted from the bathing solution, the SAP was abolished (fig. 1B). These observations, together with the fact that Co^{2+} and Mn^{2+}, but not TTX, block the SAP make it very likely that the response is due to an influx of Ca^{2+} ions[2,3]. It is therefore interesting to look for possible changes in intracellular ($[Ca^{2+}]_i$) during the SAP.

Simultaneous records of membrane potential and arsenazo absorbance from a MoG cell soma are shown in figure 2. Following a conditioning depolarization from the resting potential ($V_r = 66.9$ mV ± 5.3; $N = 59$), a long lasting action potential is elicited by a brief depolarizing pulse in preparations not treated with TEA (fig. 2A). During the SAP a rise in arsenazo absorbance is seen, which returns slowly to the baseline after the cell has repolarized. This indicates that the intracellular free Ca^{2+} level increases steadily throughout the duration of the SAP. Control records obtained at a wavelength of 570 nm (the isosbestic point of the dye)[5] demonstrated that the arsenazo Ca^{2+} signal is not appreciably contaminated by any other optical changes