β-adrenoceptor agonists both exert their positive inotropic effects by increasing I_{si} via the common mediator cAMP. In accord with this IBMX and isoprenaline have previously been shown to similarly shorten the time to peak force and the relaxation time of the isometric contraction, as cAMP elevating drugs characteristically do[12,23]. These previous findings are not contradicted by the fact that in the electrophysiological experiments presented here the shortening of the total duration of the contraction was but marginally pronounced, if at all. With the single sucrose-gap chamber used the preparations are fixed with a rubber membrane so that the relaxation of the contraction is very slow and changes in relaxation time are barely detectable (see also Reuter[18]).

There was only one point in which the effects of isoprenaline and IBMX differed. IBMX increased the cGMP content while isoprenaline did not. The IBMX-induced increase in cGMP content is probably due to the inhibition of a cGMP phosphodiesterase[24]. However, since the effects of IBMX and isoprenaline on force of contraction and on normal and slow action potentials were almost the same, we conclude that cGMP does not play an important role in mediating mechanical and electrophysiological effects of IBMX in the heart.

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Effects of oral contraceptive steroids (norethisterone/mestranol) on the activities of hepatic drug-metabolizing enzymes in iron-deficient anemic rats[1]

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Summary. Either oral contraceptive steroid (norethisterone/mestranol; N/M) treatment or iron-deficiency (Fe(–)) anemia alone caused an increase in NADPH cytochrome c reductase and in three hepatic microsomal mixed-function oxidase activities in female rats. When N/M treatment and the Fe(–) diet are combined, no further change in hepatic enzyme activity is seen compared with that with either treatment alone.
Key words. Drug metabolism; iron-deficient anemia; norethisterone/mestranol; oral contraceptive steroid.

The pharmacokinetics of many drugs have been shown to be modified following the induction of increased activity of the microsomal mixed-function oxidase (MFO) and other hepatic enzyme systems. Among these inductive factors are oral contraceptive steroids (OCS)[4] and iron-deficiency (Fe(–)) anemia[5]. In view of the frequency with which OCS treatment and Fe(–) anemia coexist in women of child-bearing age, it is important to evaluate the effect of OCS treatment on drug metabolism in the presence of such anemia. To this end, we have studied the variations of the activities of the microsomal MFO and the electron transport system in the liver of female rats treated with OCS (norethisterone/mestranol; N/M) and a Fe(–) diet, either singly or together. Data on the changes of hepatic metalloenzymes, serum lipids, and aortic glycosaminoglycan levels under the same experimental conditions have been reported previously[6,7].

Methods. Female Wistar rats weighing approximately 50 g were randomly divided into four groups of 20 animals. The control group was fed a normal diet throughout the 8-week experimental period; the N/M treated group was fed the normal diet for the first 4 weeks and then the N/M containing normal diet for an additional 4 weeks; the Fe(–) anemia group was fed the Fe(–) diet over a period of 8 weeks; and the last group for the combination study was fed the Fe(–) diet for the

Effects of norethisterone/mestranol (N/M) on hepatic drug metabolism in normal and iron deficient-anemic rats

Parameters	Groups C	C + N/M	Fe(−)	Fe(−) + N/M
Hemoglobin (g/100 ml)	16.4 ± 0.8	14.9 ± 0.6	6.99 ± 0.34[a]	6.35 ± 0.29[a,b]
Drug-metabolizing enzyme activities (nmoles/g liver/30 min)				
Hexobarbital	822.4 ± 34.8	949.6 ± 44.8[a]	1000.0 ± 20.4[a]	986.6 ± 20.6[a]
Aminopyrine	520.7 ± 17.8	644.1 ± 30.5[a]	674.9 ± 16.0[a]	703.8 ± 13.4[a]
Benzo(a)pyrene	31.37 ± 2.49	66.65 ± 9.53[a]	64.14 ± 10.11[a]	99.92 ± 30.46[a]
NADPH cytochrome c reductase (nmoles/g liver/30 min)	2026.6 ± 92.7	2341.0 ± 123.3[a]	2422.1 ± 100.5[a]	2617.0 ± 115.8[a]
Cytochrome P-450 (nmoles/g liver)	17.23 ± 0.45	19.01 ± 0.91	22.68 ± 1.13[a]	19.97 ± 0.85[a]
Microsomal protein (mg/g liver)	32.93 ± 0.84	33.72 ± 1.67	33.25 ± 1.19	32.54 ± 1.09

Values represent the mean of data from 20 rats ± SE. Abbreviations: C = control (normal) diet; Fe(−) = iron-deficient diet. [a] Significantly different ($p < 0.05$) from value for the C group. [b] Significantly different ($p < 0.05$) from value for the C + N/M group.

first 4 weeks followed by the N/M containing Fe(−) diet for an additional 4 weeks. The concentration of OCS in the experimental diets was approximately 3 mg norethisterone (progestin) and 0.15 mg mestranol (estrogen) per kg diet. The normal diet contained 50 ppm iron while the Fe(−) diet contained 6–10 ppm iron. Animals were fed ad libitum and had free access to water. Other details of the animals and the diets have been described previously[6].

At the end of the experiment, the rats were decapitated and aliquots of blood taken for hemoglobin determination by the cyanmethemoglobin method[8]. Liver microsomes prepared by the procedure described in earlier studies[9] were used for the assay of enzymes. NADPH cytochrome c reductase was assayed by the method of Williams and Kamin[10]. A radiometric method was used for the assay of hexobarbital-metabolizing activity[9]. Benzo[a]pyrene-metabolizing activity was measured by the method of Nesnow et al.[11]. The procedure of Poland and Nebert[12] was employed for the assay of aminopyrine-metabolizing activity. Microsomal protein content was estimated by the method of Lowry et al.[13]. Cytochrome P-450 was assayed by the method of Omura and Sato[14]. The significance of the differences between the various experimental groups was determined by the use of Student's t-test.

Results. The current results on the physical growth of rats (data not shown) were similar to those in an earlier study[6].

The results on drug metabolism are summarized in the table. As the mean protein content of microsomal preparations was not significantly different among the four groups, all enzyme activities and cytochrome P-450 concentrations are expressed per g liver.

Rats fed the diet containing N/M showed an increased hepatic metabolism of hexobarbital, aminopyrine and benzo[a]pyrene, and increased activities of NADPH cytochrome c reductase, while hemoglobin and cytochrome P-450 concentrations were not altered by this treatment.

Rats fed the Fe(−) diet alone showed the expected marked decline in hemoglobin levels and significant elevations in all three MFO enzymes and cytochrome c reductase activities and cytochrome P-450 levels when compared to the rats fed the normal diet.

When OCS (N/M) and the Fe(−) diet were given concurrently the values of all drug-metabolizing parameters were no greater than those observed with either treatment by itself.

Discussion. The results showing the stimulatory effects of OCS on hepatic drug-metabolizing activities in the female rat agree with the findings of other investigators[15–17], although there is some evidence in the literature that OCS treatment may result in no change, or a decrease, in oxidative biotransformations of some xenobiotics[18,19]. This increase in liver MFO activity without a change in hepatic microsomal protein content or cytochrome P-450 content agrees well with the data of others using OCS[17] or various other steroid hormones[20].

The degree of Fe(−) anemia seen in this study agrees well with our previous data[6] and was responsible for an unanticipated increase in hepatic cytochrome P-450 levels as well as stimulating a rise in MFO and NADPH cytochrome c reductase activities. These results are consistent with those recently reported by Lashneva[21] but are at variance with other studies[22] which show an increase in MFO activity without a change in cytochrome P-450 concentration. The reason for the increase of cytochrome P-450 in this study is not known, although the difficulty in producing intracellular Fe(−) in liver and a high priority of the microsomal system for the available iron have been suggested[4].

No previous studies have been reported on the combination effects of OCS and Fe(−) on the metabolism of foreign compounds. It is noteworthy that OCS (N/M) and the Fe(−) diet do not appear to have any additive effects on the values of all drug-metabolizing parameters when given together. Although it is beyond the scope of this investigation to determine the mechanism of the effects of a combination of N/M and Fe(−) anemia on the drug metabolism, the results presented here lead us to speculate that N/M and Fe(−) anemia may stimulate a similar factor necessary to initiate the production of these enzymes. However, the same results do not preclude the possibility that Fe(−) anemia rats may metabolize OCS (N/M) at a much higher than normal rats, since it has been demonstrated that OCS is metabolized in even normal animals[23], and the various drugs such as rifampicin, phenytoin and phenobarbital have enzyme-inducing properties and thus may influence the action of the OCS by increasing its metabolism[24]. Although similarities between steroid and drug metabolism are well known[25], recent studies have indicated that the steroid-metabolizing enzyme system is distinctly different from that which catalyzes drug oxidation in various tissues[26]. In addition, it has now become clear that there are many forms of cytochrome P-450 in both the steroid- and xenobiotic-metabolizing enzyme systems, each with a more or less distinctive substrate specificity and independent regulation[27,28]. Accordingly, further study is now needed to determine whether cytochrome P-450 participating in the N/M metabolism is different from that catalyzing the metabolism of drugs and xenobiotics, and whether N/M and Fe(−) anemia produce different effects on this cytochrome(s) P-450.

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Trypanosoma cruzi: metabolic labeling of trypomastigote sialoglycolipids[1]

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Summary. Trypomastigote forms (infective) of *Trypanosoma cruzi* incorporate (^{3}H)-palmitic acid and D-(^{3}H)-galactose into glycolipids. Palmitic acid-labeled acidic glycolipids were partially hydrolyzed with neuraminidase. The labeling of these compounds when the intact cell surface was labeled with galactose oxidase plus NaB^3H_4 indicates the membrane location of the sialoglycolipids.
Key words. Trypanosoma cruzi; trypomastigote forms; sialoglycolipids; neuraminidase; galactose incorporation.

Trypanosoma cruzi is the etiological agent of Chagas' disease, a chronic condition affecting approximately 20 million people in the New World. The biological cycle of the parasite is rather complex, involving at least three well-defined differentiation stages[2]. The trypomastigote stage, found in the feces of the insect vector and in the bloodstream of the vertebrate host, is particularly important since it is capable of invading vertebrate cells. The other stages are the epimastigote, found in the midgut of the insect vector and the amastigote, a dividing form encountered inside vertebrate cells. The first indications that sialic acid might be present on the surface of *T. cruzi* came from observations of the binding of cationized ferritin, and from measurements of cellular electrophoretic mobility[3]. Further work on parasite agglutination by wheat germ and *Limulus polyphemus* agglutinins reinforced this conclusion[4]. Treatment of *T. cruzi* with neuraminidase abolished the lectin-induced agglutination[4], reduced the negative surface charge[5], and stimulated the uptake of the parasites by macrophages[6]. Sialidase-sensitive molecules have also been implicated in the escape of trypomastigotes from recognition by the alternative pathway of complement, a mechanism that readily lyses epimastigote forms[7]. No data, however, were available on the nature of the sialoglycoconjugates.

Recently, sialoglycolipids have been characterized in the epimastigote forms of *T. cruzi*. The ganglioside nature of these compounds was demonstrated by the incorporation of palmitic acid into sphinganine and sphingenine in sialoglycolipids which were partially hydrolyzed by neuraminidase[9]. In the present report the existence of sialoglycolipids in the trypomastigote stage of *T. cruzi* is described. These compounds have been characterized by the incorporation of ^{3}H-palmitic acid and ^{3}H-galactose in neuraminidase-sensitive and insensitive molecules.

Materials and methods. Trypomastigotes were obtained from infected LLC-MK_2 epithelial cell monolayers maintained in Dulbecco's modified Eagle's medium (DME) containing 2% fetal calf serum (FCS)[10]. Parasites were washed once with the same medium before experimental use. Epimastigotes were cultivated as previously described[11]. Parasites (total, 4.5×10^8) were resuspended at a density of 50×10^6 cells/ml in DME containing 100 µg/ml of D-glucose, 3 µg/ml of D-galactose and 5% FCS. The cell suspension was maintained at 37°C for 30 min in a humidified atmosphere containing 5% CO_2 followed by the addition of 20 µCi/ml of D-(1-^{3}H) galactose (Amersham, 10.4 Ci/mmole; 1 mCi/ml), and incorporation proceeded for 6 h. Palmitic acid (9,10(n)-^{3}H), originally obtained in toluene (40 µl) from Amersham (500 mCi/mmole; 5 mCi/ml) was dried and resuspended in 40 µl of chloroform/ethanol (1:1, v/v). A suspension of 4.5×10^8 trypomastigotes (50×10^6 cells/ml in DME-5% FCS) was added to the radio-