Table 2. *Range of histaminolytic activity of normal pregnancy serum at intervals of 2 weeks from the 14th week onwards, as found by various investigators*

Results expressed in μg . histamine inactivated during 1 hr. incubation ($\mu\text{g./hr.}$) by 1 ml. serum.

Week of pregnancy	Ahlmark (1944) ($\mu\text{g./hr.}$)	Kapeller-Adler (1951) ($\mu\text{g./hr.}$)	Present authors ($\mu\text{g./hr.}$)
14	1.0-2.1	0.9-3.2	0.5-1.4
16	1.1-3.0	1.6-3.2	0.7-4.5
18	1.3-4.0	2.3-5.1	2.3
20	2.0-4.0	4.6-5.6	0.6-3.9
22	2.2-6.0	5.5-8.7	1.1-2.4
24	2.0-5.2	9.0-10.8	1.8-3.4
26	2.1-5.6	9.7	0.3-2.4
28	2.1-5.0	6.7-9.0	1.2-3.8
30	3.1-4.1	6.7-6.9	1.3-4.9
32	2.0-4.3	5.1-9.3	2.6-4.2
34	1.5-6.0	5.5-6.0	0.9-2.8
36	2.0-4.0	6.0-6.4	1.1-3.1
38	2.1-4.1	4.8-5.3	1.4-2.8
40	2.0-5.6	4.1-7.1	1.6-3.1

the puerperium. Ahlmark (1944) recorded levels of histaminolytic power (determined biologically) which do not differ greatly from those reported here. His figures, those of Kapeller-Adler, and ours, all expressed in μg . histamine inactivated/hour by 1 ml. serum are shown in Table 2.

There is a similar discrepancy between Kapeller-Adler's results and ours for normal placentae at full term. Whereas we found a range of 2.5 to 21 P.U./g. tissue, with an average of 9.4 P.U./g., Kapeller-Adler reported an average of about 10 times this figure, 99 P.U./g.

SUMMARY

1. When certain precautions were taken, the Kapeller-Adler technique gave consistent results in

the estimation of histaminase in pregnancy serum and in placental extracts. Attempts to develop a colorimetric modification or to use the ammonia evolved as a basis for the estimation were not successful.

2. In eighty-three cases of normal pregnancy, ranging from 14 to 40 weeks, serum histaminase varied from 0.6 to 10.6 permanganate units (P.U.)/ml., the majority lying between 2 and 6 P.U. The values bore no relation to the period of pregnancy and different cases at the same period showed wide variations.

3. In fourteen cases of normal full-term pregnancy, placental histaminase varied from 2.5 to 21 P.U./g. fresh tissue, the majority lying between 5 and 10 P.U. Extraction in the refrigerator overnight increased the yield of histaminase.

4. In four cases of toxæmia, serum histaminase values were within normal limits and the histaminase content of placenta in one toxæmia case was also normal.

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The Effect of Ergosterol and Ergocalciferol on the Anaerobic Glycolysis of Rat-liver Slices

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The exact biochemical role of vitamin D has not been established (Nicolaysen & Eeg-Larsen, 1953), but the vitamin is known to affect carbohydrate metabolism. In avitaminosis D, the rate of phosphorylation of carbohydrates is diminished and administration of vitamin D to rats leads to increased glycogen deposition in liver and muscle, as well as to an increased carbohydrate/lactic acid ratio in blood, liver and muscle (Pincussen, 1941). Zetter-

ström (1951) recently investigated the effect of adding phosphorylated vitamin D to a suspension of kidney mitochondria containing glutamate as oxidizable substrate, when an increased rate of oxygen uptake was found.

Simonsen & van Wagtenonk (1947) found that ergosterol increased the lowered anaerobic glycolysis rate of liver homogenates from guinea pigs suffering from the 'stiffness syndrome'. It has been

suggested (Festenstien, 1951) that the stiffness syndrome is analogous to hypervitaminosis D and therefore it might be expected that ergosterol and ergocalciferol would have different effects on anaerobic glycolysis. The present study was undertaken to elucidate these effects.

The initial problem was to find a satisfactory method of preparing a stable aqueous colloidal suspension of sterol, the dispersing agent of which would have a minimal or no effect on the anaerobic glycolysis. Zetterström (1951; see also Zetterström & Ljunggren, 1951), used phosphorylated ergocalciferol which is water-soluble, but in the present study, involving the free vitamin and provitamin, it was necessary to try various dispersing agents such as propylene glycol (propane-1:2-diol), Tween 80 and a mixture of lecithin and sodium stearate, which have been used by Chiu (1950), Szego (1952) and Dirscherl & Hauptmann (1950), respectively, for preparing aqueous dispersions of adrenal steroids.

EXPERIMENTAL

Experimental animals. Rats of the Wistar strain, 100–250 g. body wt., were used. They were fed on a diet consisting of 69% maize meal, 8% brewer's yeast, 8% fat, 15% milk powder with a supplement of 6 ml. hake-liver oil for every 8 lb. food.

Preparation of tissue slices. The animal was fasted overnight (usually 16 hr.), then killed by a blow on the head and bled. The liver was removed immediately and after washing in the ice-cold basic medium, was kept at 0°. Slices were cut by the method of Stadie & Riggs (1944) and suspended in ice-cold medium. A portion of the liver was blotted, weighed and set aside for drying (at 110°), to obtain the wet wt./dry wt. ratio. The slices themselves were then blotted and weighed and about 100 mg. (three or four slices) were transferred to the prepared medium (usually 1.0 ml.) in a conical Warburg flask. As each flask was prepared it was attached to a manometer and immersed in an ice bath until all the required flasks were completed.

Measurement of anaerobic glycolysis. The gas phase was a mixture of 95% N₂ and 5% CO₂ from which O₂ had previously been removed by standing over yellow phosphorus for 2 days (Burris, 1949). The flasks were filled with the gas mixture by repeated alternate evacuation and refilling (Burris, 1949). The original apparatus described for this purpose was slightly modified in that the top of each manometer was fitted with a glass tube in the form of a reversed 'h', and a glass train of T-tubes was used to join the tops of the manometer circle. 25 min. were required for equilibration instead of the usual 10–15 min.; this period was ascertained by placing thermobarometers both inside and outside the evacuation train, and it seemed that the longer period was in some way associated with the evacuation method of filling the manometric flasks. Each experiment usually involved the use of six to eight flasks. Two, outside the evacuation train, contained water and served as thermobarometers; the others contained tissue slices suspended in the basic medium with or without the test substance, an equal number of control and test flasks being generally used.

Suspending media. Bicarbonate-Ringer solution (Krebs & Henseleit, 1932) containing 0.2% (w/v) glucose was used as the basic suspending medium. Additional substances were usually added in solution and amounted to 10% of the final volume. After preparation, the media were kept at 0° and a slow stream of 95% N₂ + 5% CO₂ was passed through them. The required media were placed in the Warburg flasks just before inserting the tissue slices. $Q_{CO_2}^{N_2}$ represents μ l. CO₂/mg. dry weight/hr.

Reagents. Propylene glycol (U.S.P.) and Tween 80 (Honeywell and Stein Ltd., London) were obtained commercially. Lecithin was prepared according to the method of Pangborn (1951) and stored in ethanol under an atmosphere of N₂. Ergosterol and ergocalciferol were gifts from Glaxo Laboratories.

Absorption spectra. These were determined using a Beckman model DU spectrophotometer.

The preparation of aqueous solutions of ergosterol and ergocalciferol

Propylene glycol. Chiu (1950) used propylene glycol-water (2:1, by vol.) as a solvent for adrenal steroids and found that 0.1 ml. solvent/2 ml. aqueous medium had no effect on glycogen synthesis by rat- and rabbit-liver slices, while 0.3 ml./2 ml. medium was markedly inhibitory.

In the present investigation propylene glycol was found to inhibit the anaerobic glycolysis of rat-liver slices, the inhibition varying from 18% ($P < 0.1$) to 41% ($P < 0.01$) for 1 and 10% (v/v) propylene glycol, respectively, during the first hour.

Propylene glycol was also tested as a solvent for ergosterol by absorption-spectrum measurements on the solution. At $\lambda_{\max}$ 282.5 m μ , $E_{1\%}^{1\text{cm.}}$ = 260, and it was established that a saturated solution contained 0.027% ergosterol. On dilution of this saturated solution with 9 vol. of the Krebs bicarbonate medium, flocculation occurred and the spectrum showed considerable 'end absorption', with only very weak maxima characteristic of ergosterol. Correction for irrelevant absorption by the 'line method' of Glover (1949; see also Glover, Glover & Morton, 1952) showed that only 14% of the ergosterol originally present remained in solution.

Since a 1% solution of propylene glycol produces appreciable inhibition of anaerobic glycolysis, and when saturated with ergosterol contains only 2.7 μ g./ml. ergosterol disregarding flocculation, its use as a solvent was abandoned.

Tween 80. According to Szego (1952), 10 ml. 0.3% Tween 80 in 0.9% NaCl dissolves 25 mg. adrenal steroid.

In the present work, dispersions were prepared by heating a 0.02% solution of ergosterol in ethanol with aqueous Tween 80, under an atmosphere of N₂, until all the ethanol had been removed. Spectra were examined using compensator solutions prepared in the same way, but without sterol. The minimum concentration of Tween 80 found necessary to produce a 100 μ g./ml. solution of ergosterol showing a characteristic absorption spectrum was 0.1%.

Tween 80 inhibited the anaerobic glycolysis of rat-liver slices by 19% ($P < 0.1$) and 24% ($P < 0.05$) using 0.1% (with a female rat) and 0.01% (with a male rat) of detergent, respectively. Since 1 ml. 0.1% Tween 80 dissolves 100 μ g. ergosterol, the maximum concentration of the sterol that can be investigated without the detergent affecting glycolysis is less than 10 μ g./ml. and, therefore, as for propylene glycol, this limits the use of the solvent.

Lecithin and sodium stearate. Dirscherl & Hauptmann (1950) prepared aqueous solutions of androgens and oestrogens by adding 3 mg. of substance in 50 ml. ethanol to 500 ml. of an aqueous solution containing 0.1% sodium stearate and 0.02% lecithin. Hanahan & Wakil (1953) also used lecithin to disperse ergosterol in phosphate buffer.

In the present work, this method has been modified and sodium stearate has not been used. For a 100 $\mu\text{g./ml.}$ sterol solution, ethanolic solutions of sterol (200 $\mu\text{g./ml.}$; 2.5 ml.) and lecithin (3.6%; 0.5 ml.) were taken, water added and ethanol removed by heating under an atmosphere of N_2 . The volume was then made up to 5 ml. It was also found possible to prepare a 1 mg./ml. solution of sterol in 3.6% aqueous lecithin, although such solutions were rather viscous.

The absorption spectra of the solutions were determined using compensator solutions prepared in the same way but without sterol. Aqueous ergosterol-lecithin solutions showed appreciable non-selective absorption below 240 $\text{m}\mu$. and above 310 $\text{m}\mu$, as compared with the spectrum of an ethanolic ergosterol solution. The true absorption due to the ergosterol at 282 $\text{m}\mu$. was estimated by correcting for the irrelevant absorption by the 'line method' of Glover (1949). Of eight solutions examined, the mean amount of ergosterol in true solution was 50% (range 37–61).

The absorption spectra of aqueous ergocalciferol-lecithin solutions approximated closely to the spectrum of ergocalciferol in ethanol.

Solutions containing up to 0.36% lecithin in the Krebs medium had no effect on the anaerobic glycolysis rate of rat-liver slices during the first hour, but during a second hour variable results were obtained.

The solutions of ergosterol and ergocalciferol in aqueous lecithin were found to vary in stability, depending on their concentration: 1 mg./ml. solutions of sterol were stable for 3 days, 100 $\mu\text{g./ml.}$ solutions showed loss in intensity of absorption spectra, whereas 10 $\mu\text{g./ml.}$ solutions completely lost their characteristic absorption spectra after this time; new maxima appeared in the latter case, being at 260 $\text{m}\mu$. for ergosterol, and at 254 $\text{m}\mu$. for ergocalciferol solutions.

RESULTS

The effect of ergosterol and ergocalciferol on anaerobic glycolysis

All experiments were carried out using lecithin as solubilizing agent, and the sterol solutions were usually prepared directly before use. The results shown in Fig. 1 refer to the first hour of glycolysis. The values during the second hour were not regarded as reliable on account of the variable behaviour of the lecithin solutions during this period. The mean $Q_{\text{CO}_2}^{\text{N}}$ for all the control flasks was 0.71 (range 0.46–1.07).

The effect of ergosterol on anaerobic glycolysis of liver slices from male rats showed a decreasing inhibition with time, as shown in Fig. 2, whereas all the other effects investigated were apparently constant with time, over the period studied. The results for the effect of ergocalciferol on male animals show considerable variation within a single experiment: thus for 10 $\mu\text{g./ml.}$ the point given in Fig. 1 has been calculated using the combined

Fig. 1. Effect of ergosterol and ergocalciferol on the anaerobic glycolysis rate of rat-liver slices: the sex of the animal used is shown by the appropriate symbol; ○ represents ergosterol and ● ergocalciferol. Each point represents one rat, except for the ergocalciferol 10 $\mu\text{g./ml.}$ (male rat) point, which combines results for two animals. Three control samples and three samples + sterol were usually obtained from each animal. For points marked ○ and ● $P < 0.1$; for the others (number of points shown in parentheses) $P < 0.01$ (8), $P < 0.02$ (3) and $P < 0.05$ (4). (The three points showing no inhibition are not included here.) The more statistically significant results ($P < 0.01$ and $P < 0.02$) are all for concentrations $\geq 10 \mu\text{g./ml.}$ sterol.

Fig. 2. Progress of glycolysis of rat-liver slices in the presence of 10 $\mu\text{g./ml.}$ ergosterol: the left and right pairs of curves represent data for a female and a male rat, respectively; control values are represented by ○ and those obtained in presence of sterol by ●. For the male rat zero time corresponds to 30 min. on the scale. After 30 min. the percentage inhibition of glycolysis is 22% ($P < 0.02$) and 32% ($P < 0.01$), and after 60 min. 19% ($P < 0.01$) and 21% ($P < 0.01$), for the female and male rats, respectively. The 60 min. values are included in Fig. 1.

results of two separate animals; for the results of the single animals the inhibition is not statistically significant ($P > 0.1$ in each case). Other determinations (not shown in Fig. 1), for $0.1 \mu\text{g./ml.}$ and $0.01 \mu\text{g./ml.}$ suggested small inhibitions which were also not statistically significant ($P > 0.1$).

The spectra of ergosterol and ergocalciferol solutions after incubation with rat-liver slices

The solutions after incubation with liver slices in the usual way for 2 hr. at 38° , were filtered and examined spectroscopically. Combined lecithin solutions from the control Warburg flasks were used as compensator to examine the spectrum of the combined lecithin-sterol solutions. It was usually found necessary to dilute both solutions 20–30 times, in order to examine the spectra, since the lecithin solution itself showed strong absorption at $260 \text{ m}\mu$. compared with an unincubated solution; this was probably due to dissolved nucleic acid from the liver slices. In some cases it was found that the compensator solution showed stronger absorption than the sterol solution, with a maximum at $260 \text{ m}\mu$.; in these cases it is most likely that more nucleic acid had dissolved out in the control solutions, but it also suggests that the results to be reported must be treated with caution.

After incubation with liver slices from female rats, ergosterol solutions (100 and $10 \mu\text{g./ml.}$) still showed absorption maxima characteristic of ergosterol, though for the $100 \mu\text{g./ml.}$ solution there was also increased absorption near $290 \text{ m}\mu$., whereas a $10 \mu\text{g./ml.}$ solution used with slices from liver of a male rat showed the chief maximum at $290 \text{ m}\mu$. Two ergocalciferol solutions ($10 \mu\text{g./ml.}$) showed maxima at 250 and $295 \text{ m}\mu$. when liver slices from male rats were used, while a $10 \mu\text{g./ml.}$ solution used with liver slices from a female rat showed maxima at 260 and $320 \text{ m}\mu$., as also did a $100 \mu\text{g./ml.}$ solution using a male rat.

DISCUSSION

Both ergosterol and ergocalciferol cause inhibition of anaerobic glycolysis in rat-liver slices; this finding is in accord with the earlier observation of Pincussen (1941), that vitamin D increases the carbohydrate/lactic acid ratio in liver.

The effects of the sterols are qualitatively similar; the quantitative differences suggest that ergosterol has a greater inhibiting effect on the glycolysis of male tissues, whereas ergocalciferol has a greater effect on female tissues. It is interesting to compare these observations with those of Dirscherl & Knüchel (1950), who found that testosterone propionate and oestrone inhibited the anaerobic glycolysis rate of liver slices and diaphragms of infant mice; with testosterone, male tissues were

more affected than female, and with oestrone, female tissues were more affected than male. Both hormones were inactive at concentrations from 10^{-6} to $10^{-3} \mu\text{g./ml.}$ and $1 \mu\text{g./ml.}$ produced appreciable inhibition of glycolysis.

There is further evidence to indicate some similarity between the biochemical effects of oestrogens and ergocalciferol, and those of testosterone and ergosterol: Levrat & Brette (1951) found that gynaecomastia followed certain cases of administration of large doses of ergocalciferol, and the effects were abolished by testosterone propionate. Simonsen & van Wagtendonk (1947) also found that testosterone propionate could increase the lowered anaerobic glycolysis rate of guinea pig liver homogenates obtained from animals suffering from the stiffness syndrome. It has, however, been shown by Dirscherl, Bergmeyer & Breuer (1952), that testosterone propionate activates the anaerobic glycolysis rate of vesicular glands of mice if the rate is low, and inhibits the glycolysis if the rate is high. For the liver homogenates from guinea pigs suffering from the stiffness syndrome the rate is certainly low as compared to normal, and therefore the activation by testosterone in this case may be an effect similar to that observed by Dirscherl *et al.* (1952).

That '7-dehydrosteroids' (5:7-dienes) may have some relation to normal sexual function is suggested by the findings of Morton & Rosen (1949), confirmed by Festenstein & Morton (unpublished), of a high 7-dehydrosteroid content of frog ovaries during the reproductive phase. Feyel-Cabanes (1949), showed that pregnant rabbits after ovariectomy did not maintain pregnancy, despite progesterone injections, unless ergosterol or 7-dehydrocholesterol was also injected. Following various animal tests, neither of the 7-dehydrosteroids possessed progesterone-like, oestrogenic or androgenic activity.

The decreasing effect of the ergosterol inhibition with time, observed for male rat-liver slices, suggests that there may be some inactivation of this substance by the liver tissue. Oestrogens are believed to be inactivated by liver tissue (Paschkis & Rakoff, 1950), but Dirscherl & Hauptmann (1950) found that both oestrone and testosterone produced a constant inhibition of anaerobic glycolysis of mouse-liver slices, for several hours, provided glucose was present in the incubating medium.

The absorption maximum observed at $290 \text{ m}\mu$., after incubation of ergosterol with male rat-liver slices may be due to a product of inactivation, yet it is doubtful whether this is specific for male animals, since ergosterol solutions incubated with female tissue also show increased absorption at $290 \text{ m}\mu$.; the $295 \text{ m}\mu$. and $320 \text{ m}\mu$. maxima, found after incubation of ergocalciferol solutions with rat-liver

slices, appear for both male and female animals. Glover *et al.* (1952) found that after administration of 7-dehydrocholesterol to guinea pigs, the substance had a 'transient existence' in the liver. Tuboi (1948), perfused rabbit liver with ergosterol and 7-dehydrocholesterol, when these substances disappeared, and gave rise to cholic and deoxycholic acids in each case. These acids do not show selective absorption in the region of 290 m μ ., so that the maximum at 290 m μ . may be due to an intermediate in the conversion of ergosterol into the acids.

SUMMARY

1. The use of propylene glycol, Tween 80 and lecithin for producing aqueous solutions of ergosterol has been investigated.

2. The effect of the above substances on the anaerobic glycolysis rate of rat-liver slices has been studied: 0.01 % (v/v) Tween 80 and 1 % propylene glycol show appreciable inhibition of glycolysis, whereas lecithin up to 0.36 % has no effect.

3. Solutions of ergosterol and ergocalciferol in aqueous lecithin have been used to investigate the effect of these sterols on the anaerobic glycolysis rate of rat-liver slices; there is increasing inhibition of glycolysis with concentration, for the range 10^{-3} – 10^2 μ g./ml., ergosterol having a greater effect on male tissue, and ergocalciferol a greater effect on female tissue.

4. The spectra of aqueous ergosterol and ergocalciferol solutions have been investigated, after incubation with rat-liver slices.

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Purification and Properties of the Amine Oxidase of Pea Seedlings

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Researches extending over the past twenty-six years have established the presence in animal tissues of two oxidases, or groups of oxidases, which catalyse the oxidation of amines. Amine oxidase, or monoamine oxidase, catalyses the oxidation of aliphatic monoamines, of phenylalkylamines and of diamines of the general formula $\text{NH}_2(\text{CH}_2)_n\text{NH}_2$ with a long hydrocarbon chain. Thus Blaschko & Hawkins (1950) reported that such diamines were oxidized by amine oxidase when $n > 6$. The relative

rates of oxidation of different substrates by monoamine oxidase vary with the source of the enzyme. It is not yet clear how far this is due to variations in the specificity of the enzyme from different sources or to the presence of a mixture of enzymes. Werle & Roewer (1952) have advanced evidence for the existence in both animal and plant tissues of distinct enzymes catalysing the oxidation of aliphatic monoamines and phenylalkylamines respectively. Diamine oxidase catalyses the oxidation of diamines