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Increase in Apparent Peroxide ("Pro-blue") in Pregnant Mice

EARLIER we showed that an oral contraceptive, acting by inhibition of ovulation, decreases the total apparent peroxide in mice[1]. As a corollary to this observation it seemed worth while enquiring whether pregnancy, which also inhibits ovulation, similarly decreases peroxide.

Albino hairless mice (*hr hr*) were weighed daily, with precautions against alarm, for 3 weeks. After the first week experimentals were mated, the male being left with the female throughout the second week. Controls were unmated. Pregnancy was apparent from the increase in weight during the third week and was confirmed *post mortem*. Twelve control and twelve pregnant mice, initially 13 weeks old, were used for this experiment. Control and pregnant mice were used alternately. In three of the experiments, vaginal laves were taken from each mouse immediately after it was killed with nitrogen.

The term "apparent organic peroxide", or "peroxide" for short, refers to "pro-blue"[1], a substance or substances extractable from biological material by *n*-butanol and capable of oxidizing leucobrilliant cresyl blue in the absence of air. The peroxide estimations of whole mice and the precautions against alarm were as previously described[2]. For the peroxide estimations of mouse organs, a modified form of the anoxic box[2] was used.

Because the results of the experiment with intact mice showed a significant increase in total peroxide, separate organs were examined to determine its location. As shown in Table 1, there were significant increases of peroxide in the uterus, the ovarian fat plus ovaries and the mammary tissue. The increase in weight of the uterus plus foetuses in the pregnant mice was about 45 per cent of the total increase in weight. The lowered average weight of the ovarian fat plus ovaries in the pregnant mice suggests a utilization of ovarian fat during pregnancy, the weight of the ovaries being negligible by comparison. The mammary tissue included the skin in the area of the nipples. As far as possible a similar area of skin was taken from each mouse. The pituitary body and adrenals were also examined. The two organs were taken from the same mice but their peroxide contents were estimated separately.

Table 1. μMOLES OF PEROXIDE IN MOUSE AND ORGANS

		Mean weight	Mean peroxide	Peroxide/g
Whole mouse	Control	26·52 g	7·20	0·27
	Pregnant	29·96 g	13·38	0·45
			$P < 0·01$	
Uterus	Control	0·16 g	0·10	0·63
	Pregnant	1·73 g	0·26	0·15
			$P < 0·01$	
Ovarian fat plus ovaries	Control	0·54 g	0·14	0·26
	Pregnant	0·32 g	0·23	0·72
			$P = 0·02$	
Mammary tissue plus adjacent skin	Control	1·88 g	0·10	0·05
	Pregnant	1·80 g	0·18	0·10
			$P = 0·01$	
Adrenals	Control	4·97 mg	0·03	6·04
	Pregnant	4·87 mg	0·08	16·36
			$P = 0·01$	
Pituitary	Control		0·02	
	Pregnant		0·03	
			$P = 0·20$	

The total increase of peroxide in the organs so far tested, although statistically significant, accounts for only about 6 per cent of the whole increase. Further work is being done on other organs of pregnant mice.

It is not clear why inhibition of ovulation due to pregnancy increases organic peroxide although that due to an oral contraceptive decreases it. One obvious difference between pregnancy and oral contraception is that of hormone activity. Lipid peroxidation is believed to be associated with fatty acid metabolism[3], and pituitary activity, which is increased in pregnancy, stimulates fatty acid release.

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MICROBIOLOGY

Effect of Mixtures of Atabrine and Antibacterial Agents on the Emergence of Resistant Strains of *Mycobacterium tuberculosis*

THERE have been numerous descriptions of the successful use of mixtures of antibiotics and atabrine in preventing the emergence of resistant strains of *Staphylococcus aureus* and *Escherichia coli*[1-4]. When a sensitive strain of these organisms was subcultured in an antibiotic, however, the mixture of antibiotic and atabrine was no longer effective in eliminating resistance.

The emergence of strains resistant to chemotherapeutic agents used in the treatment of tuberculosis has narrowed the usefulness of such agents. It seemed worthwhile to investigate the effect of atabrine in suppressing the emergence of strains of *Mycobacterium tuberculosis* resistant to isonicotinic acid hydrazide (INH), dihydrostreptomycin sulphate (DHSM), and 4-aminosalicylic acid (PAS).

The H37Rv strain of *M. tuberculosis* was used. The strain, maintained on Dorset egg agar slants, was sensitive to INH, DHSM and PAS. Drug resistance was determined after the strain had been grown for 14 days at 37° C in Dubos oleic acid liquid medium. The tests were carried out by sub-inoculating 0·1 ml. of a 10^{-2} dilution of a 7 day culture in 9 ml. portions of Dubos medium containing atabrine (10 μg/ml.) and serial two-fold dilutions of INH, DHSM and PAS. Atabrine alone inhibited this strain of *M. tuberculosis* at 25 μg/ml. From the tube containing the highest concentration of the drug which exhibited growth comparable with that of the control tubes, 0·1 ml. was sub-inoculated into 9 ml. of medium containing the same and higher concentrations of the drug.

The results of this experiment are presented in Table 1. Although an increase in tolerance to the mixture of antibacteria and atabrine does eventually occur with repeated passages, there is every indication that the extent of this resistance is small compared with that obtained