

ly favorable for histological observation, while oxygen consumption measurements are independent of the amount of pigment already present in the hair bulbs at the beginning of an experiment. Independent checks on the enzymatic behavior of a given type of skin are therefore possible.

Summary. New histochemical demonstrations of tyrosinase activity in frozen sections and fragments of normal pigmented mouse skin are described. These demonstrations provide a basis for reconciling apparently conflicting results and interpretations arising from earlier studies, in which tyrosinase activity was not demonstrated, and later studies, in

which this enzyme was readily demonstrated.

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Interference Phenomenon of Browning and Gulbransen in Experimental Infection of Mice with *Trichomonas vaginalis*. (20805)

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The interference phenomenon of Browning and Gulbransen(1), *i.e.*, the antagonism of the trypanocidal dye stuff p-rosaniline hydrochloride toward 3,6-diaminoacridinium-10-methochloride (Acriflavine) has been primarily studied in trypanosomal infections with p-rosaniline resistant(1) and normally sensitive trypanosomes(2,3). According to Wright and Hirschfelder(4) the antagonism can also be demonstrated *in vitro* using the growth inhibiting activity of the dye stuffs toward yeast cells (*S. cerevisiae*). It could be expected that other organisms as long as they were sensitive to both the triphenylmethane and the acridine dye stuff would also show the interference. The pathogenicity of *Trichomonas vaginalis* for mice(5) and the response of this infection to local administration of p-rosaniline HCl and Acriflavine suggested an attempt to demonstrate the therapeutic antagonism of the 2 dye stuffs in the trichomonad infection.

Materials and methods. The strain of *T. vaginalis* was the same as that used in earlier experiments(5-7). Overnight growth in CPLM medium(8) was used in all instances. White mice from one colony were infected

subcutaneously on the ventral side with 0.5 ml of a culture diluted to contain 500000 parasites as ascertained by actual count in a hemocytometer chamber. This dose represented at least 10 minimal infective doses. After 2 hours the marked site of the infection was injected locally with 1 ml of different dilutions of aqueous solutions of p-rosaniline hydrochloride and this was followed by a local injection of 1 ml of Acriflavine solutions at the same site after another interval of 2 hours. Control series with p-rosaniline alone and Acriflavine alone as well as groups of untreated mice were included in every experiment. Four days after the infection the mice were sacrificed, autopsied, and examined microscopically for presence or absence of active trichomonads.

Experimental. It had been found in preliminary experiments that p-rosaniline HCl and Acriflavine exerted a marked activity if administered locally under the experimental conditions described above. Acriflavine is one of the most active anti-trichomonad agents which is evident from the low CD_{50} of approximately 12 $\mu\text{g/ml}$. The corresponding CD_{50} of p-rosaniline HCl was 162 $\mu\text{g/ml}$.

TABLE I. Interference Phenomenon with p-rosaniline HCl and Acriflavine in the Subcutaneous Infection with *T. vaginalis*. Infection: 500000 parasites/mouse. Therapy: Local treatment with different doses of p-rosaniline and a constant dose (25 $\mu\text{g}/\text{ml}$) of Acriflavine.

p-rosaniline, $\mu\text{g}/\text{ml}$	Acri- flavine, 25 $\mu\text{g}/\text{ml}$	No. of tests	No. of mice		% positive mice
			Neg	Pos	
100	—	6	0	48	100
100	+	6	5	43	89.6
50	+	5	6	28	82.4
25	+	4	11	17	60.7
12.5	+	4	8	24	75.0
6.25	+	2	14	2	12.5
3.125	+	2	15	1	6.25
—	+	8	53	7	11.7
—	—	8	0	56	100

The variation in this type of experiments was as a rule $\pm 30\%$.

In the interference experiments inactive doses of p-rosaniline HCl, namely 100 $\mu\text{g}/\text{ml}$ or less, were given while a constant active dose of Acriflavine, 25 $\mu\text{g}/\text{ml}$ was used. The intervals of 2 and 4 hours after the infection were selected arbitrarily but are based on earlier experience in trypanosome experiments. The results of the experiments which are compiled from 2 to 8 tests with groups of 6 to 10 mice for the different dosages are given in Table I.

From the data in Table I it is evident that inactive doses of p-rosaniline hydrochloride markedly interfered with the effect of Acriflavine 25 $\mu\text{g}/\text{ml}$, a dose which otherwise controlled the trichomonad infection in about 90% of the cases. By the preceding administration of p-rosaniline in doses of 12.5 to 100 $\mu\text{g}/\text{ml}$ the effect of Acriflavine was reduced to such a degree that dependent on the dose of p-rosaniline about 60 to 90% of the mice treated with the combination of dye stuffs showed the characteristic abscesses containing numerous active parasites. Smaller doses of 6.25 $\mu\text{g}/\text{ml}$ or less did not antagonize the effect of Acriflavine. All untreated animals showed abscesses with numerous active parasites.

These observations seemed to indicate that the interference phenomenon of Browning and Gulbransen cannot only be demonstrated in trypanosome infections but equally well in the experimental trichomoniasis of mice. In one additional experiment the effect of graded doses of Acriflavine was titrated with and without previous administration of a constant inhibiting dose of p-rosaniline hydrochloride (50 $\mu\text{g}/\text{ml}$). In presence of the antagonist the CD_{50} of Acriflavine was 35.5 $\mu\text{g}/\text{ml}$, whereas the CD_{50} of Acriflavine alone was 8.8 $\mu\text{g}/\text{ml}$. No attempt has been made in the present study to analyze the mechanism of the interference. It seemed permissible to assume that in trichomonads as it was in the case of trypanosomes(9) and yeast cells(4) the adsorption of p-rosaniline by the parasite cell prevented the binding of Acriflavine which is essential for the anti-parasitic effect.

Summary. It was demonstrated that in experiments with mice infected with *T. vaginalis* a local treatment with inactive doses of p-rosaniline hydrochloride markedly antagonized an active dose of Acriflavine. The interference phenomenon of Browning and Gulbransen applies, therefore, also to *T. vaginalis*.

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