

liver of the SM group. The spleen of the untreated control group showed complete disorganization of normal follicular pattern with a few areas of epithelioid cell infiltration but only the spleen of the SM- and not the INH-treated group, showed a few areas of mononuclear cell proliferation.

This method, therefore, detects the activity of SM and INH when the drugs are administered for 14 days only. Such simplification of screening methods in the mouse<sup>4</sup>

Effect of Isoniazid and Streptomycin Sulphate in intracardially infected tuberculous hamsters

Group	Treatment	No. of hamsters	Dose (mg/kg)	No. of doses	Mean survival time $\pm$ S.E. (days)
1	Control	7	—	—	17.3 $\pm$ 2.4
2	INH	7	5	14	30.0 $\pm$ 2.9 <sup>a</sup>
3	INH	7	50	14	51.9 $\pm$ 2.0 <sup>a</sup>
4	SM	7	100	14	49.3 $\pm$ 3.9 <sup>a</sup>

<sup>a</sup> Significantly different from control at  $P < 0.05$ .

and in the guinea-pig<sup>3,5</sup> has already been reported. This test is less costly and less time-consuming than the standard test<sup>2</sup>. Drugs of low activity like *p,p'*-diaminodiphenyl sulphone (DDS), PAS, thioacetazone (TbI/698) or their derivatives may not be detected by this rapid method<sup>6</sup>.

The need today is of 2 new drugs, completely different from INH, showing an activity equal or superior to INH as suggested by FUST<sup>7</sup>. This new screening method in hamsters is expected to show this type of activity if and when it occurs.

*Zusammenfassung.* Eine vereinfachte «screening»-Methode zur Wirkung von Isoniazid und Streptomycin wird beschrieben.

S. K. GUPTA and I. S. MATHUR

Central Drug Research Institute,  
Lucknow (India), 24 March 1969.

<sup>4</sup> F. K. FITZ PATRICK, Am. Rev. Tuberc. 77, 867 (1958).

<sup>5</sup> S. K. GUPTA and N. SEN, Indian J. med. Res. 47, 380 (1959).

<sup>6</sup> S. K. GUPTA, unpublished data.

<sup>7</sup> B. FUST, Ann. N.Y. Acad. Sci. 106, 78 (1963).

## In situ Feulgen Reaction with Schiff Reagent at Different Temperatures

In a previous study the author<sup>1</sup> has shown a progressive increase of the Feulgen staining, in the kidney nuclei of the Indian water buffalo, employing different temperatures, by a Schiff reagent whose initial pH is raised from 2.3–4.0 by a weak solution of borax. The optimum staining was found to be at 25°C. The objective of the present study is to find out the effect of different temperatures on the stainability of mammalian tissue by a Schiff reagent, prepared with basic fuchsin, at the initial pH of 2.5. This study will help to show whether or not there is any difference in staining at different temperatures between Schiff reagent used at a pH when made and that whose pH is raised to make it less acidic by a weak solution of borax. The study involves a quantitative estimation of DNA by a microspectrophotometric method. ATKINSON<sup>2</sup> has studied the in vitro reaction employing Schiff reagent and formalin at temperatures of 5–39°C. He has noted a progressive increase of the amount of regenerated fuchsin concomitant with a rise of temperature.

Schiff reagent that had an initial pH of 2.5, when prepared according to DE TOMASI<sup>3</sup>, was used in the present investigation. The reagent was prepared with basic fuchsin, made by British Drug Houses Ltd., London. The material consisted of liver of Holtzman rat. It was fixed in 40% neutral formalin for 12 h and subsequently washed in running tap water overnight. Paraffin sections, 12  $\mu$  in thickness, were used throughout the experiment. After deparaffinization, sections were hydrolysed together in 1 N HCl at 60°C for 7 min and then stained by Schiff reagent at 5, 18, 25, 30, 40, 60 and 80°C for 20 min at each temperature. Afterwards they were treated with the usual bleaching solution for 15 min, 5 min in each change. Subsequently they were dehydrated through graduated series of ethanol, cleared in dimethylaniline and then

<sup>1</sup> M. K. DUTT, Experientia 24, 1240 (1968).

<sup>2</sup> W. B. ATKINSON, Stain Techn. 27, 153 (1952).

<sup>3</sup> J. A. DE TOMASI, Stain Techn. 11, 137 (1936).

Amount of DNA at different temperatures from the liver of rat

Temperature °C	No. of nuclei	Mean nuclear diameter ( $\mu$ )	Mean DNA content with S.E.	Difference between means	t-value	P
5	20	9.85 $\pm$ 0.37	18.35 $\pm$ 1.49 (A)	A vs. B = 0.65	0.27	N.S.
18	13	9.75 $\pm$ 0.34	19.00 $\pm$ 1.94 (B)	A vs. D = 7.57	1.98	N.S.
25	12	9.50 $\pm$ 0.57	18.00 $\pm$ 2.35 (C)	B vs. D = 6.92	1.84	N.S.
30	13	9.77 $\pm$ 0.47	25.92 $\pm$ 3.26 (D)	C vs. D = 7.92	1.45	N.S.
40	20	10.07 $\pm$ 0.33	39.35 $\pm$ 2.75 (E)	D vs. E = 13.43	3.10	< 0.005
60	20	10.10 $\pm$ 0.26	38.85 $\pm$ 3.51 (F)	D vs. F = 12.93	2.41	< 0.02
				E vs. F = 1.10	1.74	N.S.

mounted in DPX, manufactured by British Drug Houses Ltd., London. Determination of the amount of DNA stained at different temperatures was made by a microspectrophotometer, described by the author<sup>4</sup>. DNA values in arbitrary units were calculated according to the method followed by the author<sup>1,5</sup>.

Microscopic examination of slides stained at different temperatures indicated that there was perfect specificity of the dye towards DNA up to a temperature of 60°C. At 80°C the specificity of the dye towards DNA is lost and the cytoplasm is also stained. The nuclear staining became weakly positive. Hence the slides stained at 80°C were not used in this study. The relevant data are presented in the Table.

From the Table it is evident that the dye-binding capacity of DNA does not increase significantly from 5–30°C or from 40–60°C. Increase of the dye-binding capacity of DNA takes place from 30–40°C. At 60°C the dye-binding capacity of DNA remains the same as at 40°C.

It is known from the experiments of ELFTMAN<sup>6</sup> that the sensitivity of Schiff reagent towards DNA increases progressively as the concentration of SO<sub>2</sub> in the reagent is lowered. The results presented here might thus indicate that at higher temperatures, viz. 40 and 60°C, optimum concentration of SO<sub>2</sub> is reached since the reagents at these temperatures attain a slightly pink colour. At 30°C the coloration is much less. At 80°C, the concentration of SO<sub>2</sub> is reached below the optimum threshold required to produce maximum reaction with the result that reactivity of the dye towards DNA is poor. The colour of

the stain at this temperature turns deep pink within 5 min, thus showing complete loss of SO<sub>2</sub>. An optimum concentration of SO<sub>2</sub> in the Schiff reagent at 40 and 60°C, therefore, causes an increase of the dye-binding capacity of the hydrolysate of DNA molecule as compared with one at lower temperatures. This interpretation is supported by the iodine titration test for the determination of the amount of SO<sub>2</sub> in the Schiff reagent, kept at different temperatures. The result of the chemical analysis reveals that a progressively greater quantity of sodium thiosulphate is required to neutralize iodine after it is made to react with starch solution. The requirement of a gradually greater quantity of sodium thiosulphate takes place at gradually increasing temperature.

*Zusammenfassung.* Mit Feulgenfärbung wurden auf Rattenleber bei Temperaturen von 40 und 60°C die besten Färberesultate erzielt.

M. K. DUTT

*Department of Zoology, University of Delhi, Delhi 7 (India), 15 January 1969.*

<sup>4</sup> M. K. DUTT, *Nucleus*, Calcutta 10, 168 (1967).

<sup>5</sup> M. K. DUTT, *Experientia* 24, 615 (1968).

<sup>6</sup> H. ELFTMAN, *J. Histochem. Cytochem.* 7, 93 (1959).

<sup>7</sup> The author wishes to express his gratitude to Prof. B. R. SESHACHAR for giving necessary facilities to carry out this investigation.

## CONGRESSUS

### Israel

#### 10th Conference of the International Society of Geographical Pathology

*in Jerusalem 1–4 September 1969*

Main topics: (1) Pulmonary emphysema; (2) Cardiomyopathies. For further information contact the General Secretary: Dr. I. S. Levij, Department of Pathology, Hebrew University, Hadassah Medical School, P.O. Box 1172, Jerusalem (Israel).

### Israel

#### 2nd International Symposium on Animal and Plant Toxins

*in Tel Aviv 22 February – 1 March 1970*

Under the auspices of the International Society of Toxicology and the Tel Aviv University the Symposium will be held in the Tel Aviv Hilton Hotel. Correspondence for further information to be addressed to: Planning Committee, 2nd International Symposium on Animal and Plant Toxins, P.O.B. 16271, Tel Aviv (Israel).

## CORRIGENDA

H. Knoche, H. Alfes, H. Möllmann and J. Reisch: *On the Biogenic Amines in the Carotid Body: Identification of Dopamine by Mass Spectrometry*, *Experientia* 25, fasc. 5, p. 515 (1969). On page 516 the authors and the address should correctly read as follows: H. KNOCHÉ, H. ALFES, H. MÖLLMANN and J. REISCH, Anatomisches Institut und Institut für Pharmazeutische Chemie der Universität, 44 Münster (Westf., Germany).

A. Pelter, R. Warren, J. N. Usmani, R. H. Rizvi, M. Ilyas and W. Rahman: *The Isolation and Characterization of*

*Two Members of a New Series of Naturally Occurring Biflavones*, *Experientia* 25, fasc. 4, p. 351 (1969). On page 352, column 2, line 32, should read correctly as follows: at 3.19 and 2.60, very little moved from the same pair...

G. Swedin and J. O. Brundin: *Distribution of Noradrenaline in the Genital Organs of the Female Rat with a Remark on Dopamine in the Cervix and Vagina*, *Experientia* 24, fasc. 10, p. 1015 (1968). In the head of the table the words *Uterine horn* and *Oviduct* should change position.