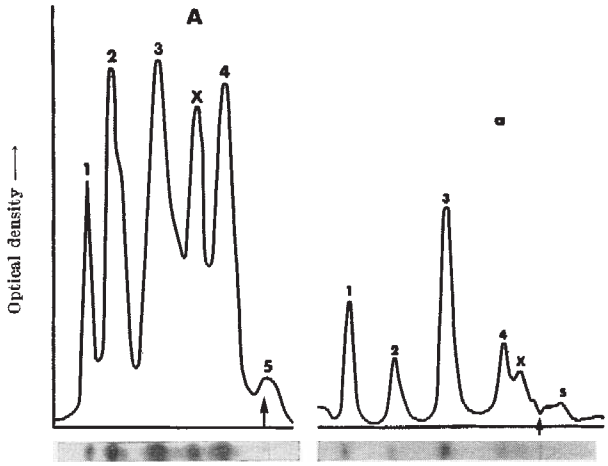

Fig. 1. Starch-gel electrophoresis of testis homogenate was conducted with the Poulik[10] buffer, *p*H 8·6, in the gel on the left (*A*), and with a phosphate-citrate buffer[12] of *p*H 7·0 in the gel on the right (*a*). In each case, migration towards the anode is to the left, and a curve of optical density against distance is shown above the stained pattern

conducted with the Poulik buffer[10], except when otherwise specified.

In a number of respects, the *X*-band exhibited characteristics similar to the other isozymes in testis. Starch-gel electrophoresis was conducted with different buffers commonly used by other investigators, at *p*H close to 8·6. It was found that the relative mobility of the *X*-band (that is, compared with the mobility of the other isozymes) was constant, despite the nature of the buffer ions. When the electrophoresis was conducted in a dilute agar medium instead of starch gel, the relative mobility was still unchanged.

Starch-gel electrophoresis of the testis homogenate was also conducted at different starch-gel concentrations, ranging from 10 to 16 per cent. The concentration of starch gel also had no effect on the relative mobility of the *X*-band. The *X*-band has the same requirements for DPN and phenazine methosulphate for demonstration of activity as other isozymes.

In some characteristics, however, the *X*-band appears to be distinct from the other isozymes. When starch-gel electrophoresis was conducted with a phosphate buffer at a *p*H of 7·0, the relative mobility of the *X*-band was altered, and it migrated between isozymes 4 and 5 instead of between isozymes 3 and 4 (Fig. 1). When a phosphate buffer at a *p*H of 8·6 was used, the *X*-band again migrated between isozymes 3 and 4.

The *X*-band also exhibited a specific resistance against 2-mercaptoethanol inhibition. One volume of the testis homogenate was mixed with one volume of a solution of 2-mercaptoethanol which had been diluted 1 to 7 with water. After 4 h incubation at 37° C, the homogenate was subjected to electrophoresis. The activity of all the isozymes was completely inhibited, except that of the *X*-band, which was unaffected.

The anomalous 6th band, previously investigated in the rat kidney, has a relative mobility which is affected by *p*H of the buffer, and a specific resistance against mercaptoethanol similar to that of the *X*-band in human testis[4]. The fact that the testis *X*-band's relative mobility is unaffected by the concentration of starch gel suggests that it is of approximately the same molecular size as that of the other isozymes in the tissue. However, the dependence of relative mobility on *p*H, and the *X*-band's resistance to mercaptoethanol inhibition, suggest the possibility that it migrates in association with some other, relatively low molecular weight substance. Such an association could account for the resistance to attack by the mercaptoethanol. The association of LDH with other substances has been observed previously[11]. It is possible that the *X*-band in human testis consists of normal *H* or *M* sub-units which is altered or complexed with some other substance.

A more complete report of this work will be published elsewhere. This work was supported by U.S. Public Health Service grant *HE*–08345–01.

N. Ressler
E. Olivero
R. R. Joseph

Department of Medicine,
Wayne County General Hospital,
Eloise, Michigan.

[1] Markert, C. L., *Science*, **140**, 1329 (1963).
[2] Cahn, R. D., Kaplan, N. O., Levine, L., and Zwilling, E., *Science*, **136**, 962 (1962).
[3] Conklin, J. L., Dewey, M. M., and May, B., *J. Histochem. Cytochem.*, **10**, 365 (1962).
[4] Ressler, N., Cook, U., Olivero, E., and Joseph, R. R. (in preparation).
[5] Blanco, A., and Zinkham, W. H., *Science*, **139**, 601 (1963).
[6] Zinkham, W. H., Blanco, A., and Kupchyk, L., *Science*, **142**, 1303 (1963).
[7] Zinkham, W. H., Blanco, A., and Kupchyk, L., *Science*, **144**, 1353 (1964).
[8] Ressler, N., Schulz, J. L., and Joseph, R. R., *Nature*, **197**, 872 (1963).
[9] Ressler, N., Schulz, J. L., and Joseph, R. R., *J. Lab. Clin. Med.*, **62**, 571 (1963).
[10] Poulik, M. D., *Nature*, **180**, 1477 (1957).
[11] Cellers, P. G., Stock, A., and Pfleider, G., *Biochem. Biophys. Acta*, **77**, 577 (1963).
[12] Kaplan, N. O., and Cahn, R. D., *Proc. U.S. Nat. Acad. Sci.*, **48**, 2123 (1962).

Shikimic Acid, a Constituent of Tutsan Berries

The ripened seed capsules of tutsan (*Hypericum androsemium*) were dried, ground and extracted with petroleum ether (60°–80° C) followed by further extraction with acetone. The residue from the evaporated acetone extract, on vacuum sublimation, yielded a colourless solid, which crystallized from acetone–petrol in colourless plates m.p. 184°, with analysis C, 48·1; H, 5·8 per cent. $C_7H_{10}O_5$ requires C, 48·2; H, 5·8 per cent. Preliminary tests showed unsaturated and acidic properties. The infra-red spectrum proved to be identical with that of shikimic acid[1] (*cyclo*hex-1-ene-3 : 4 : 5-triol-1-carboxylic acid) which is recorded in D.M.S. spectrum 2635.

K. R. Hargreaves

Dundee Technical College,
Scotland.

[1] Fischer, H. O. L., and Dangschat, J., *Helv.*, **20**, 705 (1937).

New Types of Kinins and their Action on Fruit Tree Species

We have been engaged in work on the effects of some kinetin analogues on various tree species and have found that they have somewhat different biological activity[1,2]. Because of the comparatively low solubility of 6-furfurylamino- and 6-benzylaminopurine in water, which complicates the application of these compounds, our interest was directed primarily to the preparation of kinetin derivatives with higher solubility in water. The solubility of kinins can considerably influence their transport and therefore also their practical application. After application of 6-benzylaminopurine-8-^{14}C into the petioles of leaves of one-year-old apple shoots, the applied kinin was found by autoradiography about 10 cm in the basipetal direction from the point of application whereas kinin transport in the apical direction was negligible[1,2]. The most soluble compounds of this type synthesized by us up to now are *N*-(6-purinyl)-β-phenylalanine and *N*-(6-purinyl)-α-phenylglycine and the 6-benzylamino-9-glucosylpurine already described[3,4]. The first of these had no biological activity. 6-Benzylamino-9-glucosylpurine was about as active as 6-benzylaminopurine, whereas the biological activity of *N*-(6-purinyl)-α-phenylglycine depended on the

type of application. The activity of *N*-(6-purinyl-α-phenylglycine applied to the bark and bast is more active in stimulating the buds of apple trees from the winter vegetative rest than is 6-benzylaminopurine itself, or 6-benzylaminoglucosylpurine[5]. Fig. 1, where *N*-(6-purinyl)-α-phenylglycine was applied to every second bud, shows that leaves of considerable size shot from the stimulated buds within 20 days, whereas no change was observed in the unstimulated control buds.

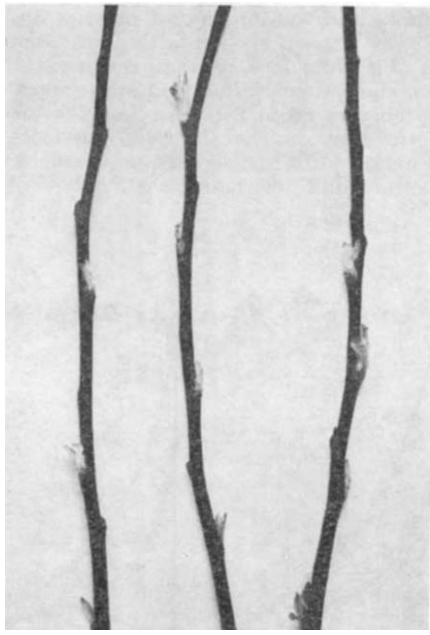

Fig. 1

When disks of leaves were cultivated no damage was observed within a week at 20 γ/ml. concentrations of an aqueous *N*-(6-purinyl)-α-phenylglycine solution, whereas the edges of the disks were necrotized in identical concentrations of 6-benzylaminopurine.

N-(6-purinyl)-α-phenylglycine and *N*-(6-purinyl)-β-phenylalanine were prepared by condensation (3 h) of 6-chloropurine with α-phenylglycine or β-phenylalanine and in the presence of sodium hydrogen carbonate in methyl 'Cellosolve' at 120°–130° C. The product was recrystallized from 90 per cent acetone and chromatographed on Whatman No. 1 paper in the system acetic acid–butanol–water; R_F for $C_{13}H_{11}N_5O_2$ = 0·64.

For $C_{13}H_{11}N_5O_2$ (269·2), m.p. 230°–232° C (49 per cent), calculated: 26·01 per cent N; found: 25·47 per cent N. For $C_{14}H_{13}N_5O_2$ (283), m.p. 305°–308° C (decomp.), yield 54·5 per cent, calculated: 59·35 per cent C; 4·62 per cent H; 24·72 per cent N; found: 58·92 per cent C; 4·42 per cent H; 25·10 per cent N.

J. Beneš
K. Vereš

Isotope Laboratory,
Institutes of Biological Research.

L. Chvojka
A. Friedrich

Institute of Experimental Botany,
Czechoslovak Academy of Sciences,
Prague.

[1] Chvojka, L., Vereš, K., and Kozel, J., *Biol. Plant.*, Praha, **3**, 140 (1961).
[2] Chvojka, L., and Vereš, K., *Proc. V. Intern. Biochem. Congr.*, Moscow (1961).
[3] Brown, G. B., and Weliky, V. S., *J. Biol. Chem.*, **204**, 1019 (1953).
[4] Blackburn, G. M., and Johnson, A. W., *J. Chem. Soc.*, 4347 (1960).
[5] Chvojka, L., Kračmar, P., Beneš, J., and Belea, A., *Biológia*, **8**, 579 (1963).

PHYSIOLOGY

Effects of Cross-union of Motor Nerves to Fast and Slow Skeletal Muscles

The principal difference in the dynamic properties of fast and slow skeletal muscles of the rat has been attributed to differences in their force : velocity properties[1]. If this difference were maintained by specific neural influences[2,3] the primary effect of cross-union of motoneurones would be a change in the intrinsic speed of shortening of the contractile material. According to the view that the active state duration is dependent on the intrinsic speed of shortening[1], this should lead to a change in the isometric twitch contraction time which is inversely proportional to the change in speed. Furthermore, there should be no change in the twitch : tetanus ratio resulting from these changes in the force : velocity properties because the same mechanical events would take place on an altered time scale. A series of examinations is being made to test these possibilities and to determine the effects of nerve cross-union at different times after the operations. The results described below were obtained from rat extensor digitorum longus (EDL) and soleus (SOL) muscles 30–47 weeks after cross-union of their motor nerves.

Operative cross-union[3] of the nerves to EDL and SOL muscles was performed on three-week-old female rats (Wistar) in aseptic conditions; the anaesthetic was 50 mg pentobarbital sodium/kg body-weight injected intraperitoneally. In four animals the nerves to EDL and SOL were transected and cross-united in the right leg. In two of these animals (Fig. 1, *B* and *D*) the same nerves in the left leg were transected and self-united; in the other two animals (Fig. 1, *A* and *C*) no operation was performed on the left leg. The dynamic properties of these muscles were determined using the methods and apparatus described previously[1]. All measurements were made with the muscles at 35° C.

Fig. 1 shows representative records of isometric responses of normal, self-innervated and cross-innervated muscles. Records of contractions were obtained from either a normal or a self-innervated muscle in one leg as controls for the responses of the cross-innervated muscle in the other leg. Comparison of the responses of normal (*N*-EDL, *N*-SOL) and self-innervated (*S*-EDL, *S*-SOL) muscles shows that there is virtually no change resulting from transection of the nerves and subsequent re-innervation. In contrast the responses of cross-innervated muscles (*X*-EDL and *X*-SOL) are greatly altered and assume a form similar to that which characterized the muscle formerly innervated by the nerve. In comparison with control muscles, the cross-innervated EDL muscles receiving SOL nerve (*X*-EDL) show an increase in the times for contraction and half-relaxation, and increased summation of clonic contractions at 20 c/s (Fig. 1 *A* and *B*). The reverse changes are evident in records of contractions of cross-innervated soleus (*X*-SOL) muscles (Fig. 1 *C* and *D*). Values for the contraction time (Tc) and half-relaxation time ($T\frac{1}{2}R$) are given in Table 1. Furthermore, there are changes in the form of isometric tetanic contractions following nerve cross-union. The rate of development and decay of isometric tetanic

Table 1. Characteristics of Isometric Contractions and the Constants describing the Force : Velocity Properties of Normal, Self-innervated and Cross-innervated Extensor Digitorum Longus (EDL) and Soleus (SOL) Muscles of the Rat at 35° C. Abbreviations are defined in the Text

Animal	Age (wks.)	Muscle	Muscle weight (mg)	Sarcomeres per fibre	Tc (msec)	$T\frac{1}{2}R$ (msec)	P_t/P_0	P_0 (g)	Vs when $P=0$ (μ/sec)	a/P_0
A	50	*N*-EDL	145	4,287	13	7·5	0·197	372	48·5	0·285
		X-EDL	87	4,189	27	41	0·243	76	26·4	0·185
B	33	*S*-EDL	130	4,138	15	9·25	0·197	189	41·6	0·33
		X-EDL	80	3,813	27	38	0·242	28	25·15	0·152
C	46	*N*-SOL	140	4,724	36	55	0·186	190	22·4	0·14
		X-SOL	165	6,254	16	18·5	0·197	129	34·6	0·265
D	40	*S*-SOL	146	5,513	41·5	64	0·235	182	17·65	0·17
		X-SOL	146	6,241	18	22	0·19	206	36·5	0·285