Table II—Vasoconstrictor-Hypertensive Activity

No.[a]	Dose, mg./kg. i.v.	Nasal Volume: Δ mm.[3]	Nasal Volume: Duration, min.	Blood Pressure: Δ mm. Hg	Blood Pressure: Duration, min.	Blood Pressure: Dose, mg./kg. i.v.	Blood Pressure: Change, %	Blood Pressure: Duration, min.
III								
a			[b]			10	+25	5
b	1.0	+300	6	+15	6			
c	1.0	+500	14	+45	5			
d	5.0	+80	14	−25[c]	>14			
e	1.0	+240	10	+5	5	10	+22	45
f	1.0	+580	17	+50	>17			
g	0.5	+680	9	+20	9			
h	1.0	+600	13	+20	13	10	+100	45
i	5.0	+530	36	+37	20			
Phenylephrine hydrochloride	0.01	733	9	+30	9			

[a] Refers to compound numbers in Table I and in the text. [b] Nasal volume changes were not measured for this compound. [c] Doses of 10 to 100 mg./kg. did not produce consistent blood pressure effects.

tracheotomy performed. A femoral artery was cannulated for blood pressure recording. A 22.9-cm. (9-in.) metal rod fitted with a small rubber ball was passed into the dog's mouth to the soft palate so that the ball pressed against the wall of the nasopharynx. A glass nose cannula was then inserted into a nostril, and a clamp was applied to hold the cannula in place and to seal the other nostril. The cannula was connected to a Grass PT-5A volumetric low pressure transducer, and the volume (pressure) changes of this cavity were measured. In some experiments only blood pressure was monitored. All compounds were administered intravenously

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Gas Chromatographic Determination of Chlorphenesin in Plasma

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Abstract □ A procedure is described for the determination of chlorphenesin in plasma. Chlorphenesin is extracted from plasma with chloroform, reacted with bis-(trimethylsilyl)-acetamide, and the silylated derivative is measured quantitatively by gas chromatography. The technique is reproducible and accurate in the range of 1–10 mcg./ml. Dibutyl phthalate is used as internal standard for quantitation by the relative peak height method.

Keyphrases □ Chlorphenesin in plasma—determination □ Plasma, analysis—chlorphenesin □ GLC—analysis □ Dibutyl phthalate—internal standard

Chlorphenesin (3-*p*-chlorophenoxy-1,2-propanediol) has been shown to suppress immunological response in a number of animal systems (1–3) and to inhibit the release of histamine from sensitized human leucocytes by ragweed antigen (4). Although chlorphenesin has been known since 1949 and a number of procedures have been described in the literature for the analysis of chlorphenesin (5, 6) or chlorphenesin carbamate (7), none are sufficiently sensitive to measure the presence of chlorphenesin in biological fluids after therapeutic doses. Procedures for the quantitative analysis in plasma of a closely related compound, mephenesin [3-(*o*-tolyloxy)-1,2-propanediol], have been reported by Titus *et al.* (8) and by Wyngaarden *et al.* (9). However, these analyses depend upon diazotization or nitration of mephenesin at the *para* position of the phenol ring and are not applicable to chlorphenesin which contains a chlorine atom at this location.

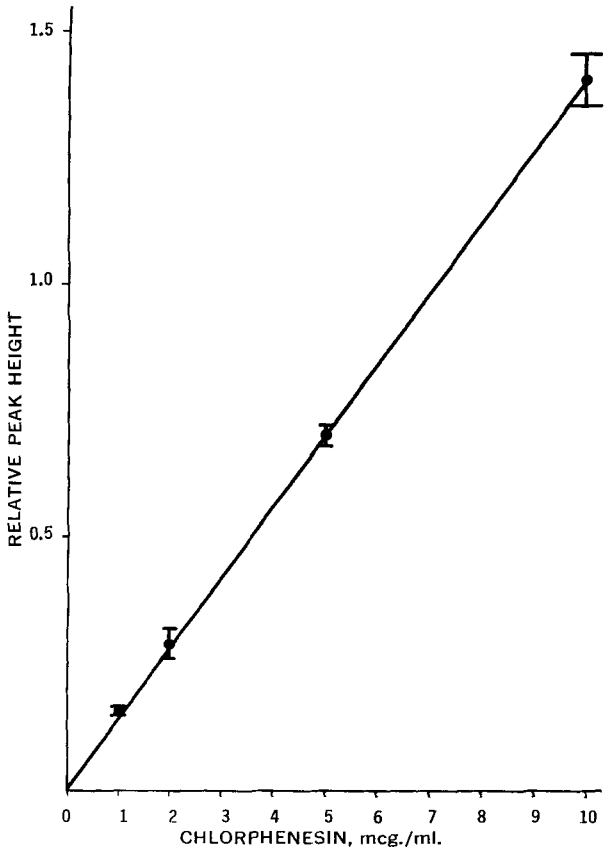

Figure 1—*The relationship between relative peak height and concentration of chlorphenesin in plasma.*

This manuscript describes a procedure for the quantitative determination of chlorphenesin in plasma.

EXPERIMENTAL

Equipment and Reagents—A F and M model 402 dual column gas chromatograph equipped with a hydrogen flame ionization detector and a 1-mv. Minneapolis-Honeywell recorder were employed. The chromatographic columns used were 91.4-cm. × 0.635-cm. (3-ft. × 0.25-in.) glass tubes packed with 5.0% DC200 on 80–100 mesh diatomaceous earth[1]. The instrument settings were as follows: temperature-column, 170°; injection port, 275°; detector block, 230°. Gas flow rates—hydrogen, 60 ml./min; helium (carrier gas), 38 ml./min; oxygen, 70 ml./min. Sensitivity settings were range 10 with an attenuation factor of 8×. The reagents were redistilled chloroform, dibutyl phthalate (Eastman), and bis-(trimethylsilyl)-acetamide (Applied Science).

Procedure—Plasma, 0.5 ml., was diluted with 0.5 ml. of saline and the fluid was thoroughly shaken with 0.5 ml. of redistilled chloroform. Following centrifugation, 0.2 ml. of the chloroform layer was transferred to a small centrifuge tube and treated with 20 μl. of bis-(trimethylsilyl)acetamide for 1 hr. at room temperature. The reaction mixture was evaporated to dryness in a stream of nitrogen, and the residue was taken up in 20 μl. of redistilled chloroform containing 100 mcg./ml. of dibutyl phthalate. This chloroform solution (2.6 μl.) was injected into the gas chromatograph, and the concentration of chlorphenesin was determined by the relative peak height method using dibutyl phthalate as internal standard.

[1] Chromosorb WAW, Johns Manville Products Corp., New York N. Y.

RESULTS AND DISCUSSION

It was necessary to convert chlorphenesin to a suitable derivative for gas chromatographic analysis since its chromatogram showed two peaks. Chlorphenesin can be quantitated gas chromatographically when relative peak height is used as an index of concentration since a linear relationship exists between relative peak height and drug concentration (trimethylsilyl ether derivative of chlorphenesin) in the range of 1–10 mcg./ml. of plasma (Fig. 1). The reproducibility of the procedure is indicated by the standard error of the triplicate determinations shown in Fig. 1.

The extraction procedure effectively separates chlorphenesin from normal interfering plasma constituents since determinations in normal human plasma give little or no blank (<0.5 mcg./ml.). It was also established that the known major metabolites of chlorphenesin, *p*-chlorophenoxylactic acid and *p*-chlorophenoxyacetic acid (10), do not interfere in the assay procedure.

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