# THE PHARMACOLOGICAL ACTIONS OF THE CRYSTALLINE PRINCIPLES OF *AMMI VISNAGA* LINN.

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ATTEMPTS to isolate the crystalline principles of the fruit of *Ammi Visnaga* (in Arabic "Khella") date since 1897, when Mustapha[1] obtained by extraction with alcohol and ether, white silky needles, bitter in taste and sparingly soluble in water. Mustapha gave this substance the name of khellin. The work was continued by Malosse[2], Fantl and Salem[3], Fahmy and El-Keiy[4] and Samaan[5]. The crystalline substances obtained by these authors were, in many instances, not subjected to a satisfactory chemical analysis, the proof of their purity resting mainly on a rough examination of their physical properties. The small amount of consideration which most of these authors had paid to the results obtained by the preceding workers led to a considerable confusion of nomenclature; different names were frequently given to apparently the same substance. A similar confusion exists also in the pharmacological literature concerning *Ammi Visnaga*, different properties being sometimes attributed to the same crystalline substance.

The recent work of Späth and Gruber[6,7,8] placed the problem on a sound chemical basis. These authors isolated from the fruit of the plant three crystalline substances which on analysis proved to belong to the group of chromones. In a private communication to one of us (G.V.A.) Prof. Gruber states that, in addition to the three chromones, he had also detected the presence of a small amount of a coumarin, the analysis of which he has not, however, yet completed. Therefore, until further proof is available, only the three chromones, so far isolated and analysed, are to be considered.

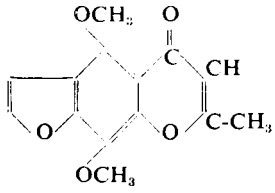
According to Späth and Gruber, these substances are:—

(1) *Khellin*, isolated by Mustapha and by Fantl and Salem and shown by Späth and Gruber[6] to be a dimethoxymethylfurano-chromone (I) m.pt. 154°C.

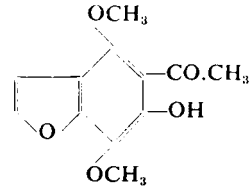
(2) *Khellol glycoside*, isolated by Fantl and Salem and shown by Späth and Gruber[8] to be a monomethoxymethylfuranochromone, oxyglycoside (II) m.pt. 175°C.

(3) *Visnagin*, isolated by Späth and Gruber[7] and shown by them to be a monomethoxymethylfuranochromone (III) m.pt. 144°C.

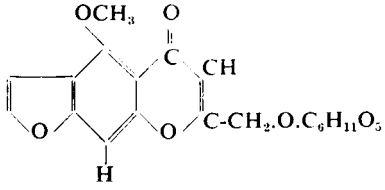
It can be seen from the structural formulæ given that the simplest compound is visnagin, that khellin is a methoxyvisnagin and that the khellol glycoside is an oxyglycoside of visnagin. Taking the visnagin radical as R, khellin is R-$OCH_3$ and khellol glycoside R-$OC_6H_{11}O_5$.



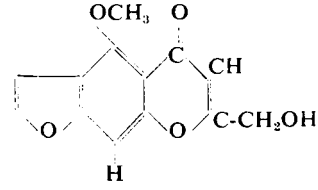
I. Khellin



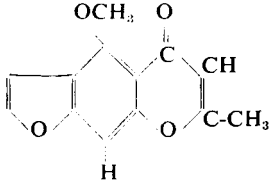
IV. Khellinon



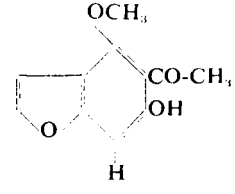
II. Khellol glycoside



V. Khellol



III. Visnagin



VI. Visnaginon

Späth and Gruber have also analysed a large number of derivatives of these compounds. The most interesting were obtained by the action of alkali and of acids. By alkali, khellin is split to a dimethoxyacetylhydroxy coumarone (m.pt. 99° to 101°C.) and visnagin to a monomethoxyacetylhydroxy coumarone (m.pt. 109° to 111°C.). The authors gave the name of khellinon to the first compound (IV) and visnaginon to the second (VI). The aglycone obtained by the hydrolysis of the khellol glycoside in acid was first isolated by Fantl and Salem[3], who gave it the name of khellol (m.pt. 179°C.). Späth and Gruber showed that the khellol is a hydroxyvisnagin (V). It was further shown that the glycoside, as well as the khellol, when treated with alkali give a product which is in all respects identical with visnaginon. In a simplified form the relations between these compounds are as follows:

Khellin is converted by alkali into khellinon
Khellon glycoside is converted by acid into khellol

Khellol glycoside, Khellol, Visnagin } are all converted by alkali into visnaginon

The decoction of the dried fruit of *Ammi Visnaga* has been used since ancient times by the population in the Middle East, and is frequently prescribed by the local physicians as a diuretic and as an anti-spasmodic in cases of ureteral stones. Samaan[9] investigated the pharmacological

action of khellin and of the glycoside (which he respectively called visammin and khellinin) and found that khellin increases the flow of urine and causes a relaxation of the visceral plain muscles. The interest in the crystalline constituents of *Ammi Visnaga* has been recently revived as the result of the demonstration by Anrep, Barsoum, Kenawy and Misrahy[10] that khellin causes a conspicuous dilatation of the coronary blood vessels without much affecting the systemic circulation. The interest in the substance was further stimulated by the promising results of the clinical observations upon the effect of khellin in the anginal syndrome (Kenawy and Barsoum[11], Anrep and *et al.*[122] Ayyad[13]).

Salama[14] showed in animals and man that none of the crystalline principles of *Ammi Visnaga* exerts a diuretic action, the increased formation of urine being entirely due to the fluid taken in the form of the decoction.

Samaan[15,16] ascribes the coronary vasodilator action of *Ammi Visnaga* not to khellin, but to the glycoside, which, according to him, causes in concentrations of 1 in 25,000 and even 300,000 a conspicuous increase of the coronary outflow of the isolated perfused rabbit's heart. In experiments in which an artificial spasm of the coronary blood vessels was induced by barium chloride, administration of the glycoside was stated to cause sometimes as much as a twelve-fold increase of the coronary outflow. On the other hand, according to Bagouri[17], the glycoside, even when used in high concentrations, exerts no action on the coronary vessels of the perfused heart, the coronary vasodilatation being entirely due to khellin.

It follows from the above that further research is required before a proper assessment of the pharmacological potency of the different crystalline principles of *Ammi Visnaga* can be made. We, therefore, undertook to investigate the comparative action of khellin, of visnagin, of the glycoside and of their derivatives by quantitative methods.

## Methods of Preparation

The three natural crystalline substances of *Ammi Visnaga*, khellin, khellol glycoside and visnagin were prepared according to the method devised by Späth and Gruber. The substances were repeatedly crystallised from methyl alcohol and other solvents until their respective melting-points reached the maximum values given by the Austrian observers and did not change by further recrystallisation. The purity of the final products was controlled in the Pharmacognosy Laboratory and in the Faculty of Science of this University. The fission products, khellinon, visnaginon and khellol were obtained by the action of acid and alkali as recommended by Späth and Gruber. The purification of these substances presents no difficulty since they easily crystallise from methyl alcohol and give sharp melting-points, 100°C. for khellinon, 110°C. for visnaginon and 179°C. for khellol.

## THE COLORIMETRIC AND THE BIOLOGICAL ASSAY OF THE CRYSTALLINE PRINCIPLES OF *Ammi Visnaga*

*Colorimetric assay:*—The moderately stable pink coloration which khellin gives, as discovered by Fahmy and El-Keiy[4], in contact with solid sodium hydroxide, served as the basis for the colorimetric assay of khellin and of visnagin. For quantitative work we used a saturated solution of potassium hydroxide and a 0·5 millimolar standard solution of khellin in water (0·13 mg. of khellin per ml.). Addition of 0·1 ml. of the khellin solution to 1·0 ml. of saturated potassium hydroxide solution gives an intensity of colour suitable for work with a sensitive colorimeter of the ordinary type or with a photoelectric colorimeter. As has already been shown by Fahmy, this method is less suitable for the estimation of the glycoside, which, in presence of strong alkali, gives a very unstable cherry-red colour. The products of acid and alkaline hydrolysis of the three natural substances give no pink coloration in presence of strong alkali.

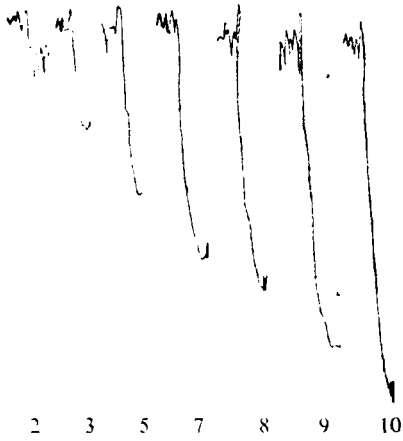



FIG. 1. Response of the rectal cæcum of the fowl to gradually increasing doses of khellin. The amounts administered are shown in μg.

*Biological assay:*—The biological method of assay used in this work was originally devised by Barsoum and Gaddum[18] for the estimation of adenosine. As a test object they used the rectal cæcum of the fowl suspended in Tyrode's solution in a bath 5 ml. in capacity. We find that the rectal cæcum can be satisfactorily used also for the comparative assay of the active principles of *Ammi Visnaga*. With sensitive preparations it is possible to make the assay with an accuracy of about 10 per cent. Figure 1 shows the reaction of the rectal cæcum to gradually increasing doses of khellin which, for this purpose, was dissolved in Tyrode's solution. An average preparation of the rectal cæcum is sensitive to about 2 μg. of khellin, i.e., a concentration of khellin in the bath of $2{\cdot}5 \times 10^{-7}$. Figure 2 shows that repeated administration of the same dose of khellin gives the same degree of relaxation of the rectal cæcum, and Figure 3 serves as an example of a comparative assay of visnagin and of khellin showing that visnagin is about 30 per cent. less active than khellin.

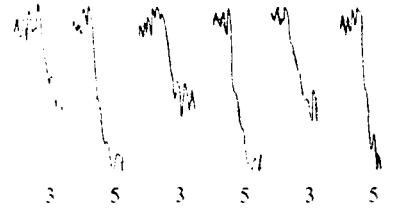
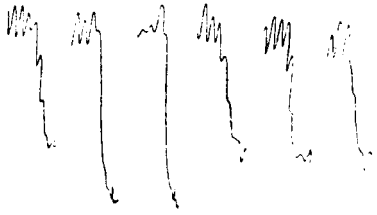



FIG. 2. Regular response of the rectal cæcum to alternate doses of 3·0 and 5·0 μg. of khellin.

Table I gives the results obtained with the three natural substances and with the products of their hydrolysis. The results of the colorimetric and of the biological assays given in Table I are means of not less than 50 separate estimations of the 3 natural substances and of their derivatives. Usually only 2 or 3 substances were assayed on one rectal cæcum. Before administration, the solutions were warmed to the temperature of the bath containing the cæcum. The doses of the substances were such as to cause 30 to 50 per cent. of the maximal relaxation of the intestinal muscle, which is the range of maximal discrimination of the preparation.



10 μg. Visnagin 10 μg. Khellin 15 μg. Visnagin 10 μg. Khellin 7 μg. Visnagin 10 μg.

FIG. 3. Comparative assay of khellin and of visnagin on the rectal cæcum of the fowl. The amounts of the two substances administered are given in μg.

TABLE I

COLORIMETRIC AND BIOLOGICAL ASSAYS OF KHELLIN, OF VISNAGIN, OF KHELLOL-GLYCOSIDE AND OF THE PRODUCTS OF THEIR HYDROLYSIS. ALL THE SUBSTANCES WERE PREPARED IN 0·5 MILLIMOLAR SOLUTIONS AND KHELLIN WAS TAKEN AS THE STANDARD FOR THE COMPARISON

EQUIVALENT DOSES IN μg.

| | Khellin m.pt. 154° C | Visnagin m.pt. 144° C | Glycoside m.pt. 175° C | Khellinon m.pt. 100° C | Visnaginon m.pt. 110° C | Khellol m.pt. 179° C |
|---|---|---|---|---|---|---|
| Colorimetric assay ... ... | 10 | 14·5 | not tested | gives no colour reaction | gives no colour reaction | not tested |
| Standard deviation ... ... | — | 0·5 | — | — | — | — |
| Biological assay ... ... ... | 10 | 15 | over 300 | 30 | 50 | 40 |
| Standard deviation ... ... | — | 0·6 | — | 1·1 | 2·2 | — |
| Percentage of activity in relation to that of Khellin | | | | | | |
| | 100 | 66 | traces | 33 | 20 | 25 |

Table I reveals the following points of interest:

(1) Colorimetric and biological assays show that the monomethoxy derivative, visnagin, is about 30 per cent. less active than the dimethoxy derivative, khellin. The agreement between the colorimetric and the biological assays is extremely satisfactory.

(2) Khellinon, the alkali-split product of khellin, loses its colour reaction, but partially retains its power to relax the smooth muscle. Biologically, khellinon is about one third as active as khellin.

(3) The biological action of the glycoside, as tested on the rectal cæcum, is not less than 30 times weaker than that of khellin. In fact it is questionable whether it has any action at all.

(4) Khellol, the product of acid hydrolysis of the glycoside, shows a considerably greater activity than the mother substance. Apparently the substitution of the glycoside radical by the hydroxyl group partially unmasks some of the latent activity of the rest of the molecule.

(5) Khellol is a monomethoxy derivative which differs from visnagin by containing a hydroxyl group. This difference is sufficient to cause a conspicuous diminution of the activity of khellol as compared with that of visnagin.

(6) Visnaginon, similarly to khellinon, gives no colour reaction. Its biological action is about 30 per cent. weaker than that of khellinon.

The action of khellin on the rectal cæcum is considerably weaker than that of adrenaline, 4 $\mu$g. of khellin being approximately equivalent in action to 0·01 $\mu$g. of adrenaline chloride. Khellin is, however, about 12 times more effective than aminophylline. The rabbit's uterus is also relaxed by khellin, especially when it has previously been contracted by administration of adrenaline, showing that khellin acts directly on the plain muscle and not on the sympathetic nerve endings.

### The Comparative Action of Khellin, of Visnagin and of the Khellol Glycoside on the Coronary Circulation

At the time when Anrep and *et al*[10] made their observations upon the coronary vasodilator action of khellin in the heart lung preparation, the existence of the related monomethoxy compound, visnagin, was not yet known. So far, visnagin does not present much interest from the practical point of view, since it occurs in the fruit of *Ammi Visnaga* in very small amounts. However, in the future it might possibly be prepared synthetically. From the theoretical point of view it presents a greater interest because a comparison between visnagin, khellin and the glycoside might throw a light on the relation between the action of these substances and their molecular structure. The comparison of the action of khellin and of the glycoside on the one hand and of khellin and of visnagin on the other, was made on the standard heart-lung preparation on dogs by collecting the blood through a coronary sinus cannula. A few typical experiments, selected from amongst many others, are sufficient to illustrate the action of these substances.

*Experiment* 1. Heart-lung preparation; systemic output 650 ml./minute; aortic blood pressure 120 mm. Hg. For about 20 minutes the coronary outflow remained constant at 42 to 44 ml./minute. After a gradual administration of 40 mg. of the glycoside the coronary blood flow remained unchanged. Administration of 5 mg. of khellin increased it to 59 ml./minute; after another dose of 5 mg. of khellin the flow increased to 90 to 95 ml./minute. The total amount of blood in circulation was about 800 ml.

*Experiment* 2. Heart-lung preparation; output 500 ml./minute; aortic blood pressure 120 mm. Hg. The outflow of blood from the coronary sinus remained constant for over 15 minutes at 58 to 61 ml./minute. 4 doses of the glycoside, 20 mg. each, were administered at intervals of a few minutes; 80 mg. in all. The coronary blood flow remained unchanged although the drug was allowed to circulate for several minutes. Administration of 10 mg. of khellin rapidly increased the coronary blood flow to 120 ml./minute. The total amount of blood in circulation was about 700 ml.

*Experiment* 3. Demonstrated to the Cairo Clinical Society. Heart-lung preparation output 450 ml./minute, arterial blood pressure 130 mm. Hg. The outflow from the coronary sinus was 51 ml./minute. On administration of 45 mg. of the glycoside the coronary outflow remained the same. After administration of 15 mg. of khellin it increased to 250 ml./minute. The amount of blood in circulation was about 500 ml.

The action of khellin was extremely prolonged, the coronary blood flow remaining increased to the end of an experiment. In this, the effect of khellin greatly differs from that of amyl nitrite.

In some of the experiments the action of khellin on the coronary blood flow was more and in others less conspicuous than in the above examples. As regards the glycoside, no coronary vasodilator action could be demonstrated in the heart-lung preparation, even though its concentration was increased to 100 $\mu$g./ml.

We were also able to confirm the observation of Bagouri[17], who found that systemic blood vessels are much less sensitive to the vasodilator action of khellin than coronary blood vessels, and that the glycoside caused no coronary dilatation in the perfused rabbit's heart.

The glycoside, since it has no action on the coronary blood vessels, could be administered together with khellin in the same heart-lung preparation. This is not possible when comparing the action of khellin with that of visnagin. Both are coronary vasodilators, the action of which persists for a very long time. The action of the two drugs was, therefore, studied on two separate preparations which were made to work in, as nearly as possible, the same experimental conditions. The type and the weight of the dogs used for the two preparations were the same; the arterial blood pressure, temperature and the output of the heart were respectively maintained at the same levels, and the two hearts usually did not differ in weight by more than 5 g. In spite of these precautions, the individual variations of the coronary sinus outflow were too large to permit of a definite conclusion as regards the relative action of the two drugs. The observations made on the rectal cæcum would suggest that visnagin might possibly be a somewhat weaker coronary vasodilator than khellin. Observations made on two separate heart-lung preparations are not, however, sufficiently accurate to justify this conclusion. As compared with aminophylline the action of khellin on the coronary circulation in the heart-lung preparation is 4 to 6 times stronger.

## Observations on the Alimentary Tract *in situ*

The experiments were made on dogs of 7 to 9 kg. weight, anæsthetised with chloralose, sodium luminal or nembutal. The abdomen was opened by a median incision and a loop of the jejunum, about 30 to 40 cm. in length, was tied off between two ligatures. A wide cannula was inserted into each end of the loop. The two cannulæ were then connected to a separating funnel the top of which was joined to a volume recorder. The funnel, filled with saline solution, was kept at

a suitable height, sufficient to fill but not to distend the intestinal loop. The contractions of the loop were recorded on a drum. Intravenous injection of khellin in doses of 5 to 10 mg. caused a rapid and conspicuous relaxation of the intestinal loop, the rhythmic movements being

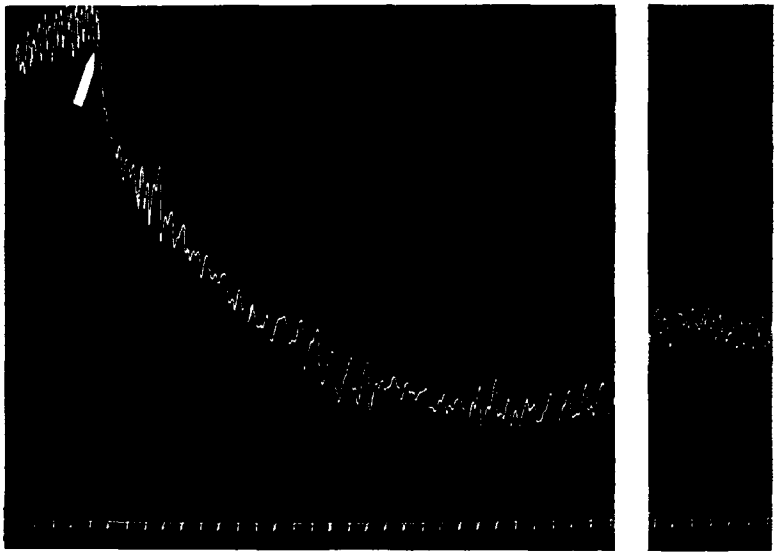


FIG. 4. Dog 10 kg. Left, effect of intravenous injection of 10 mg. of khellin on the intestinal movements recorded as described in the test. Right, 20 minutes later. Time in 10-second intervals.

reduced in rate and in strength. The relaxation of the intestine following administration of khellin was extremely prolonged, the recovery being slow and usually incomplete even after 1 to 2 hours. Figure 4 shows the effect of administration of khellin on the intestinal movements in the whole animal. Khellol glycoside even in greater doses caused no relaxation of the intestinal loop and no diminution of the rhythmic contractions (Fig. 5). In some experiments injections of the glycoside were followed by an increase of the intestinal tone.

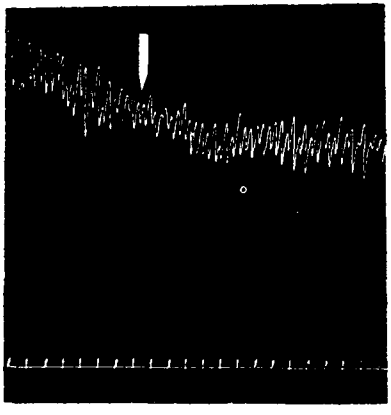


FIG. 5. Dog 9·5 kg. Effect of injection of 20 mg. of khellol glycoside on the intestinal movements. Times in 10-second intervals. The intestine was slowly relaxing before the injection.

## OBSERVATIONS ON THE BRONCHIAL MUSCLES

Samaan[9] showed that khellin causes a relaxation of bronchial muscle, but did not study the effect by quantitative methods. Our ex-

periments were performed on perfused lungs of the guinea-pig, using the method of Sollmann and Oettingen[19]. The lungs were suspended in an air thermostat at a temperature of 38° to 40° C., the air being kept saturated with water. A cannula was introduced into the trachea, and the lungs were perfused with warm oxygenated Ringer-Locke solution in the same manner as the isolated heart. In order to allow an outlet, the surface of the lung was scarified in several places, the fluid being collected through a wide funnel in a graduated cylinder. All the measurements were made without opening the thermostat so as to avoid cooling the lungs. The results of some of the experiments are given in Table II.

TABLE II

ACTION OF KHELLIN ON THE BRONCHI OF THE PERFUSED LUNGS OF THE GUINEA-PIG

| Initial flow in ml./minute | Concentration of khellin in mg./l. | Maximum flow in ml./minute | Percentage increase |
|---|---|---|---|
| 5·0 to 5·4 | 10 | 42·0 to 43·0 | 700 |
| 4·4 to 4·8 | 10 | 39·0 to 39·6 | 750 |
| 2·5 to 2·6 | 6 | 11·6 to 12·0 | 350 |
| 3·3 to 3·5 | 4 | 9·8 to 10·2 | 195 |
| 3·5 to 3·7 | 2 | 5·2 to 5·4 | 47 |

Other similar experiments gave approximately the same results. The minimum effective concentration of khellin causing a 50 per cent. increase in the outflow was about 2 to 3 μg./ml. After replacing the khellin solution with Ringer-Locke solution the outflow of the fluid from the bronchial tree usually returned to its original volume in the course of 4 to 5 minutes. The effect of khellin is strong enough to antagonise considerable concentrations of histamine. When the lungs are perfused with 10 mg./l. of khellin, injections of 5 to 10 μg. of histamine diphosphate have only a negligible effect. In absence of khellin these doses cause a conspicuous broncho-constriction. The glycoside even in high concentrations causes no relaxation of the bronchi. As a broncho-dilator khellin is 4 to 6 times more effective than aminophylline.

As a result of the above observations administration of khellin was tried in a large number of patients suffering from bronchial asthma. With a few exceptions, presented by subjects with advanced emphysema or fibrosis of the lungs, khellin was extremely beneficial. The asthmatic attacks were cut short, the vital capacity of the subjects increased and the feeling of respiratory distress disappeared. On continuous administration of khellin the attacks either disappeared or became less frequent and less severe. The treatment of bronchial asthma with khellin will form the subject of another communication.

### ESTIMATION AND DISTRIBUTION OF KHELLIN IN BLOOD AND TISSUES

The estimation of khellin in blood and tissues can be made by the colorimetric method or by the biological method on the rectal cæcum. The following procedure was adopted for the preparation of the final extracts suitable for quantitative estimations. Ten ml. of blood was

added to 100 ml. of alcohol; after filtration the precipitate was washed with three quantities of alcohol, each of 10 ml. The washings and the filtrate were mixed and evaporated on a water-bath, under reduced pressure, to a volume of about 5 ml.; 50 to 100 ml. of water was then added and the solution was treated in a separating funnel with 3 quantities of chloroform, each of 10 ml., to extract the khellin. The chloroform solution was evaporated to complete dryness and the residue was dissolved in exactly 1 or 2 ml. of distilled water, for the colorimetric test, or, of Tyrode's fluid, for the biological assay. This method presents the advantage that the khellin content of any reasonable quantity of blood or of other biological fluids can be concentrated in the 1 or 2 ml. of the final extract. The colorimetric or the biological assay was made against a standard 0·5 millimolar solution of khellin. The recovery of khellin by the above method is 95 to 100 per cent. When the method is used for tissues, a piece of an organ is weighed, ground with silver sand, treated with alcohol and extracted with chloroform as described for blood. Control observations showed that the extraction of khellin from tissues is somewhat less complete, ranging between 85 and 95 per cent. Extraction of khellin from fat is less satisfactory, khellin being highly soluble in lipoids. The accuracy of the colorimetric and of the biological method is the same.

*Distribution of khellin in blood.*—Khellin added to defibrinated blood or to blood rendered incoagulable by addition of heparin is taken up by the serum or plasma and by the red blood corpuscles. With concentrations varying between 1 and 200 $\mu$g./ml. the plasma or serum contained about 10 to 20 per cent. more khellin than the red blood corpuscles. This proportion is not changed by allowing the blood to stand for several hours before it is centrifuged. It is well known that the red blood cells are able to take up a large number of organic substances, some of which are easily given off, while others become fixed and, therefore, probably biologically inactive. Glucose, for example, belongs to the first group of substances and histamine to the second. We find that khellin is rapidly given off by the corpuscles when these are exposed to serum or Tyrode's solution containing no khellin. It is, therefore, obvious that the khellin of the red blood corpuscles is not pharmacologically wasted. It should be looked upon as a store which is readily released to the surrounding plasma.

After intravenous or intramuscular administration of khellin the drug at first appears in the blood in a high concentration. Within a few minutes the khellin concentration begins to diminish, and in about 20 to 30 minutes it reaches a steady level which is maintained for several hours. The rapid diminution of the khellin concentration in the circulating blood is not due to excretion by the kidneys or to destruction by the tissues, but to a gradual and more or less uniform distribution of the drug amongst all the organs of the body.

The drug remains in the circulation for an extremely long time. In dogs after injections of 10 to 20 mg./kg. khellin could be detected in

the blood as long as 36 hours later; 24 hour-samples of urine collected after the injection contain only traces of khellin. The conclusion must be, therefore, made that the khellin is not eliminated by the kidneys in an unchanged form.

Repeated administrations of khellin lead to its accumulation in the blood and tissues. Animals injected with doses of 10 mg./kg. had a concentration of 4 $\mu$g./ml. of blood, 24 hours after the first injection and 12 $\mu$g./ml. 24 hours after the ninth injection. A similar accumulation of the drug can be also demonstrated in man. For example, in subjects who received one injection of 200 mg. of khellin per day its concentration in the blood, 30 minutes after the first injection, was 4 to 5 $\mu$g./ml. and after the 5th injection, 12 to 17 $\mu$g./ml.

In order to study the distribution of khellin in the tissues the drug was injected intramuscularly in doses of 20 to 40 mg./kg., the administration of such large doses being necessary since only small samples of tissues could be used to obtain a perfect extraction. The dogs were killed at different intervals of time after the injections and their tissues analysed. In one set of experiments the first animal was killed 1 hour after the injection, the second 24 hours, and the third 36 hours later. The concentration of khellin in the blood of these animals was 30, 16 and 5 $\mu$g./ml. respectively. The concentration in the liver, muscle, brain, kidney and the mesentery varied between 20 and 40 $\mu$g./g. 1 hour after the injections, between 7 and 15 $\mu$g./g. 24 hours and between 2 and 5 $\mu$g./g. 36 hours later. It follows that khellin is rapidly distributed over the whole body. The concentration in the liver was always somewhat higher than in the other tissues. The figures obtained for the brain were the lowest, which is probably due to the difficulty of extraction of khellin from lipoid-containing tissues. The disappearance of khellin from the tissues is extremely slow and is not related to any particular organ.

### Absorption of khellin and of the khellol glycoside from the alimentary tract

Khellin is absorbed from the stomach, from the small intestine and from the large intestine. The absorption from the stomach and from the small intestine was studied in anæsthetised dogs after complete separation of the pylorus from the duodenum. Khellin solutions were injected directly into the stomach or into the duodenum. In some of the experiments khellin was injected into a separated loop of the small intestine. Absorption from the large intestine was studied only in man. The blood samples were collected after the respective injections and assayed for khellin in the usual manner. The absorption from the alimentary tract is not followed by a temporary large increase of the khellin concentration in the blood as is the case with intramuscular absorption. Oral administration is, therefore, suitable for the maintenance of a high concentration of khellin in the blood, while intramuscular

administration is more suitable when it is desired to raise its concentration in a short time.

In man, after oral administration of 300 mg. the maximum concentration of 5 to 6 $\mu$g./ml. of blood was reached in 40 to 60 minutes. After rectal administration of 500 mg. dissolved in 50 ml. of alcohol (20 per cent.), the maximum concentration of 6 to 8 $\mu$g./ml. was reached in about 2 hours. It can be seen that the rate of absorption of khellin from the large intestine is not inferior to that from the rest of the digestive tract.

No evidence could be found to show that the khellol glycoside is converted in the body to active khellin. After oral or intramuscular administration of 4 to 5 g. of the glycoside, in non-anæsthetised dogs, no khellin could be detected in their circulating blood. Neither is there any evidence showing that the glycoside is absorbed from the intestinal tract. In dogs, anæsthetised with chloralose, a loop of the small intestine, about 50 cm. long, was tied off and its two ends were provided with cannulæ. After thoroughly washing the inside of the loop, 70 ml. of a solution containing 1 mg./ml. of khellin or of the glycoside in alcohol (20 per cent.) was injected into the loop, which was then closed and returned to the abdominal cavity. After 30 to 90 minutes the loop was emptied into a measuring cylinder and washed 2 or 3 times with alcohol (20 per cent.) to remove all traces of the injected substance. The amount of khellin or of the glycoside which escaped absorption was then determined. The khellin was determined as described before for blood and the glycoside, by measuring the reducing power of the intestinal content before and after hydrolysis in acid following a preliminary precipitation of the protein matter with alcohol (95 per cent.). The results of 4 experiments are given in Table III.

TABLE III

ABSORPTION OF KHELLIN AND OF KHELLOL GLYCOSIDE FROM THE SMALL INTESTINE AMOUNT OF FLUID, ALCOHOL (20 PER CENT.), INJECTED INTO THE LOOP WAS IN EACH CASE 70 ML. CONTAINING 70 MG. OF KHELLIN OR OF THE GLYCOSIDE

| Substance injected | Duration of absorption in minutes | Amount of fluid not absorbed ml. | Amount of substance not absorbed mg. |
|---|---|---|---|
| Khellin ... ... ... ... | 30 | 43 | 14 |
| Khellin ... ... ... ... | 30 | 41 | 17 |
| Glycoside ... ... ... ... | 30 | 48 | 68 |
| Glycoside ... ... ... ... | 90 | 26 | 72 |

## CONCLUSIONS

1. A biological method of assay and a modification of the colorimetric method of assay of the active crystalline principles of *Ammi Visnaga* are described.

2. A comparative colorimetric and biological assay of khellin, of the khellol glycoside and of visnagin showed that the glycoside is biologically almost inactive and that the activity of visnagin is about one-third less than that of khellin.

3. The glycoside has no detectable action on the coronary circulation.

4. No difference could be detected between the coronary vasodilator action of khellin and of visnagin.

5. Kellinon and visnaginon, the products of alkali hydrolysis of khellin and visnagin respectively, give no colour reaction, but still exert some biological activity. The action of khellinon is about one-third that of khellin, and the action of visnaginon about one-third that of visnagin.

6. Khellol, the product of acid hydrolysis of the glycoside, has a definite biological action which is much stronger than that of the glycoside and is about 25 per cent. of that of khellin.

7. Khellin causes a conspicuous relaxation of the bronchi in the isolated lungs and a diminution of the intestinal tone in the whole animal. The khellol glycoside is in these respect inactive.

8. Khellin is rapidly absorbed from the small intestine, from the stomach and from the large intestine.

9. Intramuscular injections of khellin are followed by a rapid and conspicuous increase of its concentration in the circulating blood, which after some time gradually diminishes and finally becomes stabilised at an approximately uniform level. Oral administration is not followed by such a temporary large increase; the concentration of khellin increases gradually and reaches a stable level in about 30 minutes.

10. Khellin is not excreted in the urine in an unchanged form and it disappears from the blood and tissues at a very slow rate.

11. In man, oral or intramuscular administration of a single dose of 100 to 200 mg. of khellin raises its concentration, in the blood to above the minimal effective concentration, which has been shown to cause a coronary vasodilatation and relaxation of the bronchi. Due to the slow destruction of the drug, repeated administration leads to its accumulation in the body.

12. No evidence could be found that the khellol glycoside is converted in the body to khellin or that it can be absorbed from the intestine in an unchanged form.

## REFERENCES

1. Mustapha, *C. R. Acad. Sci., Paris,* 1879, **89,** 442.
2. Malosse, *Thesis. Montpellier,* 1881.
3. Fantl and Salem, *Biochem. Z.,* 1930, **226,** 166.
4. Fahmy and El-Keiy, *Rep. Pharm. Soc., Egypt,* 1931, **13,** 36.
5. Samaan, *Quart. J. Pharm. Pharmacol.,* 1931, **4,** 14.
6. Späth and Gruber, *Ber. dtsch. chem. Ges.,* 1938, **71,** 106.
7. Späth and Gruber, *ibid.,* 1941, **74,** 1492.
8. Späth and Gruber, *ibid.,* 1941, **74,** 1549.
9. Samaan, *Quart. J. Pharm. Pharmacol.,* 1932, **5,** 6.
10. Anrep, Barsoum, Kenway and Misrahy, *Brit. Heart. J.,* 1946, **8,** 171.
11. Kenawy and Barsoum, *Gaz. Fac. Med., Cairo,* 1945, **13,** 39.
12. Anrep, Barsoum, Kenawy and Misrahy, *Lancet,* 1947, **252,** 557.
13. Ayyad, *Lancet,* 1948, **254,** 308.
14. Salama, *Gaz. Med. Fac. Cairo,* 1946, **13,** 10.
15. Samaan, *Quart. J. Pharm. Pharmacol.,* 1946, **19,** 135.
16. Samaan, Hossein and Ridi, *ibid.,* 1947, **20,** 502.
17. Bagouri, *J. Pharm. Pharmacol,* 1949, **1,** 177.
18. Barsoum and Gaddum, *J. Physiol,* 1935, **85,** 1.
19. Sollmann and von Oettingen, *Proc. Soc. exp. Biol., N.Y.,* 1928, **25,** 692.