Sanguinarine & hypothalamic glaucoma

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Here is a story, a true story which can rival any tale of fiction, one chapter of which began nearly four hundred years ago when Elizabeth the First was the Queen of England. In the city of London there lived a famous herbalist John Gerard, who grew beautiful flowers gathered from all parts of the world. In that epoch of adventure and discovery one of his friends brought for him a few black seeds from the West Indies, which must have looked very much like our Indian mustard seeds, and they blossomed in his Bloomsbury garden into a beautiful yellow poppy with silver-veined but prickly leaves. This American poppy, first described in Gerard's 'Herbal', was soon introduced to Oxford, Paris and other European gardens. Because of its orange latex, it was wrongly identified with 'argemone', a Mediterranean poppy with orange juice, reputed to cure "eye diseases" in ancient Greek classical medicine, and baptised Argemone mexicana Tournefort (1694), Linnaeus (1753), [Figure - 1], the suffixes in honour of the great taxonomists. This was a most unfortunate mistake, for this American weed neither came from Mexico nor does it possess the reputed eye-curing virtues of the hitherto unknown 'argemone' herb. Probably because of tais mistaken is identification and spurious reputation, the plant was deliberately introduced by the Portuguese into Africa and Goa in Western India.

The weed spread like magic and soon covered vast areas of Africa, India, South-East Asia, China and Australia. It is one of the most prolific and common weeds in the eastern tropics and all of us here have seen entire fields covered by this yellow prickly poppy, growing from the winter to the rains. It is specially important to emphasise to an Indian audience that this American plant was unknown in India and deliberately

introduced into this country only during the last two hundred years and that it cannot therefore, be equated with any plant of ancient Ayurveda. With its ill-fated destiny for misbaptism, the new arrival, because it yielded a "golden milk", was falsely identified in India with 'Svarnakshiri', an obsolete Ayurvedic healing herb. Ainslie, in his first (western) pharmacopoea of Indian drugs, as far back as 1813, recorded that our Vaidyas and Hakims freely prescribe the plant and seeds for diseases of the skin and eyes.

Argemone seeds are both accidently and deliberately used to adulterate mustard seeds (Indraji, 1910) and their seed-oil to adulterate other edible seed-oils. The argemone stem is cooked and eaten as a salad in many parts of Bengal and Bihar.

Argemone seed-oil is used as a purgative in the Mauritius Islands, Abysinnia, South Africa and India and for colic, cholera and sedation in our country. The seeds are used as a narcotic and for diarrhoea and dysentry, the herb as a diaphoretic, expectorant, diuretic, and application for eczema and ophthalmia.

The herb and its seed-oil are prescribed in many modern Ayurvedic works (Jadavji, 1950) and are easily available to people in our villages. As the Honorary Secretary to the Bombay Government Committee on Research in Ayurveda, I interviewed and corresponded with a large number of practising Vaidyas. It was alarming to find most of them wrongly insisting that Argemone mexicana L., is an indigenous Indian therapeutic herb, and some advocating its poisonous oil as a laxative.

Epidemic-dropsy Glaucoma & World Glaucoma

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Records are available from 1860 of epidemics of dropsy occurring in India and affecting large groups of persons. Between 1866 and 1954, thirty epidemics are officially recorded. Glaucoma associated with Dropsy was recorded several times after 1909. In the 1935 epidemic, 7,000 people were affected, over 700 developed glaucoma and 1,500 died. Our Indian workers in Bengal first obtained the clue that oil presses had been used to extract argemone seed-oil and subsequently for extraction of sesame or other cooking oils. Persons ingesting the contaminated cooking oils developed symptoms of the disease. These observations of Sarkar and Kamath in 1926 and 1928 completed a seventy-year epoch of search for the cause of the devastating epidemics of dropsy and blindness. S. N. Sarkar, my predecessor at Oxford, working under Sir Rudolph Peters, showed that argemone seedoil contained two alkaloids, sanguinarine and dihydrosanguinarine, and tried to explain some of the clinical observations on the biochemical blockage of pyruvate metabolism by sanguinarine. I took up pharmacological studies with sanguinarine from several different aspects and succeeded in producing both dropsy and rise of eye tension in experimental animals. (Hakim, 1953, 1954, Duke-Elder, 1954).

At that stage it seemed obvious that the Indian epidemics of dropsy and glaucoma and records of argemone poisoning in animals were caused mainly by the toxic alkaloid sanguinarine present in argemone seed-oil. The next problem was whether some of the glaucoma occurring in Europe and America could also result from ingestion of some insidious toxins. To establish any such international hypothesis, dual evidence would be required that sanguinarine or similar toxins occur in these non-tropical countries and

that they can be available to man. This hypothesis, which seemed so fantastic in 1953, is now well within the range of probability, as I have been fortunate in finding multiple and rich sources of sanguinarine in most parts of the world, as well as several routes by which this blinding poison could reach man.

Sanguinarine & Other Eye Tension Raising Alkaloids in Plants

Sanguinarine is a benzphenanthridine alkaloid belonging to the iso-quinoline group. Over 114 iso-quinoline alkaloids are known and some of them like morphine, codeine and papaverine are amongst the most active and useful of drugs. Iso-quinoline alkaloids are usually found in plants belonging to two large botanical families, the poppies and fumarias, but they also occur sporadically in twelve other plant families (Manske, 1954). Sanguinarine was hitherto regarded as a rare and obscure alkaloid first isolated 133 years ago from Sanguinaria canadensis L., the 'blood-root' used by the North American Indians to paint themselves scarlet. During the last one and a half centuries, the "epoch of alkaloid detection", chemists found sanguinarine in only eight other poppy-fumaria plants. Working at the National Institute for Medical Research, London, under the guidance of Sir Robert Robinson and Dr. James Walker, we found that sanguinarine was present and abundant in most morphological parts of nearly fifty species of poppy-fumaria weeds we examined [Table - 1]. All that was required was a few milligrams of plant material ground in acetic acid, separated either by paper chromatography or electrophoresis and compared with standard alkaloids. Sanguinarine, and at times ten other related alkaloids, were separated from a few drops of this simple extract and showed striking and beautiful fluorescent colours under filtered ultra-violet light. Sanguinarine was more or less invariably present in the leaves and stems, often in the roots, capsules and seeds, and was the commonest fluorescent alkaloid seen in these species. It seems to have been missed for so many years because of its unusual solubility and absence of adequate .tests. The details of our methods of extraction, detection, identification and results have been published elsewhere. (Hakim et al., 1961a).

An example of how sanguinarine had hitherto escaped chemical detection is found in the 'opium' poppy Papaver somniferum L. No plant has been more thoroughly investigated in the annals of chemistry, and yet sanguinarine was missed in this plant. We found it in its roots, stems and leaves. The young plants are used as edible herbs in the middle East and like the 'fumitory' herb, deliberately fed to cattle.

You will realise a little later on the purpose of this elaboration into the phyto-chemistry of sanguinarine. At this stage, I am trying to bring out the widespread source of sanguinarine in plants, firstly because a few of these are directly ingested by man, and secondly, as I shall show later, a large number of such plants grow as prolific weeds in most parts of the world *(Fedde,* 1909; Hutchinson, 1921), are grazed upon by cattle, who absorb their toxic alkaloids, store them in their liver and secrete them in their milk.

Those of you who have seen the cornfields of Europe and North India will recollect the myriads of scarlet poppies *(Papaver rhoeas L.*, P. *hybridum* L., and P. dubium L.) growing amongst the grain. Everyone of these poppies contain sanguinarine in either its roots, stems, leaves or seeds. The poppies are collected with the harvest and contribute

their poisons both to the grain and to the dried fodder which is fed to cattle.

On this table is a vase filled with the Indian *'fumitory'* (Fumaria indica Pugsley), an elegant weed I collected this morning from the fields of Amritsar. It grows throughout the Indo-Gangetic plain and on the hills of South India and is very similar to the soecies that grow abundantly throughout Persia, the Middle East, Europe and America. Shakespeare (1523) described the European species ([Figure - 2]-Fumaria officinalis L.) as growing wild and prolific in the fields of France. The roots and seeds of both species are rich in sanguinarine. I wish to draw your special attention to this herb as it is likely to be responsible for a considerable number of cases of endemic rather than epidemic glaucoma in our country, throughout the Middle East, and in other parts of the world.

The 'fumitory' herb has been widely used as a medicine, and must have contributed an insidious source of sanguinarine during its recorded history covering over 2,000 years! This herb was known as 'Kaonos' in ancient Greek classical medicine and prescribed by Dioscorides and Pliny. Under its Arabic na-ne of 'Shahtiraj', it was used by Ibn Seranion, Ibnu'l-Baytar, Ibn 'Awwam, 'Abd ar-Razzaq, Rabi Maimonides and others and its use continued throughout the Middle East unto the present day. As 'Shahterah, it was well-known to the physicians of Persia, Ar-Razi, 'Ali ibnu'l 'Abbas al-Ma usi, Abu 'Ali Sina and Isma'il Jurjani and its use was introduced into the Mughal. school of Yunani medicine in lnaia through works like the Makhzanel-Adwia. The herb is well known in North India as 'Sathra' (often confused with 'Pitpapda'), and is commonly used as a Yunani and even as an Ayurvedic decoction for fevers, skin and kidney diseases, blood-purification etc. Our hostess, on watching me collect the herb this morning, strongly recommended it for treatment of "women's diseases" (? !). So deep-rooted is this ancient tradition that not only Shakespeare but practically every ancient and modern herbal throughout Europe, extolls the virtues of the European 'fumitory' and of several species of poppies. Even the National Formulary (1960), the Dispensatory of the United States of America (1960) and the British Pharmaceutical Codex (1949) sponsor the use of *Sanguinaria canadensis* L., the 'blood-root' overflowing with the scarlet alkaloid sanguinarine !

Several orange-red stems and roots were prescribed as direct applications for the eyes in ancient empirical systems of medicine. These are usually from poppy-fumaria or closely related genera. The oldest extant of Ayurvedic texts - the Bower Manuscript - re-commends the application of 'rasot', an extract of berberis. Dioscorides used 'argemone', probably a thalictrum. The famous 'mamiran', from *coptis, corydalis or thalictrum,* was prescribed by Al-Ghafiqi in Spain, and is extensively used, even at present, in Afghanistan and North India.

The first link connecting sanguinarine to its possible aetiology in world glaucoma, was the chemical investigation I have described earlier. It became evident that sanguinarine was not a rare and obscure alkaloid, but a glaucomagenetic toxin definitely found and abundant in most parts of at least sixty-two poppy-fumaria species [Table - 1]. Since the alkaloid was present in so many species distributed in 14 representative genera, it seems very likely that it will be found in most or all of the nearly seven hundred species of this vast plant family. If you visualise rice fields in spring, teeming with thousands of yellow argemone, or vegetable gardens scattered with the purple fumaria in early winter, or fields of wheat amongst which thousands of scarlet poppies fill an autumn landscape, you will realise how densely just three out of these hundreds of species can

grow and, therefore, what an abundant source of sanguinarine is available in the plant kingdom.

The next significant discovery was that the eye tension-raising action of sanguinarine on experimental animals is not the unique property of this alkaloid, but that several similar alkaloids also produce acute rise of eye tension in experimental animals. My early Oxford experiments had shown that, besides sanguinarine, chelerythrine and a number of crude poppy extracts when injected subconjunctivally or intra-ocularly in rabbits and monkeys, raised their eye tension. Lieb and Scherf from Prof. Thiel's group at Frankfurt, later showed that seven out of fourteen iso-quinoline alkaloids, injected intravenously into anaesthetized rabbits, produced acute rise of eye tension. When these alkaloids were later available to me through the kindness of Dr. Manske, I produced acute rise of eve tension by their sub-conjunctival injection.

Whilst working at the National Institute for Medical Research, I found a new and very interesting technique of raising eye tension by injecting minute doses of sanguinarine into the brain of conscious cats. The very first injection of sanguinarine given by this method in 1957, raised eye tension it was also significant that several other iso-quinoline alkaloids, besides sanguinarine, when injected into the brain also produced acute rise of eye tension [Table - 2]. As these other alkaloids, sent by Dr. Manske, belong to twelve plant families, besides poppy-fumarias, it is interesting to speculate if iso-quinoline alkaloids found in species of spinach, for example, or others found in the custard-apple or the Indian bael-fruit, or even in oranges and limes, may have some subtle insidious action in the aetiology of widespread glaucoma.

Transmission of Sanguinarine to Man Through Milk

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It is of little practical importance to know that sanguinarine and other eye tension raising alkaloids are abundantly and widely distributed in plants growing in all parts of the world. The whole crux and importance of the experiments is the demonstration that these toxins reach and affect man. From the beginning, I realised that contamination of cooking oils by argemone oil could not explain the large number of endemic cases of human glaucoma in India or glaucoma in other parts of the world where the argemone plant does not flourish. Inquiries among my ophthalmological colleagues suggested that the Joins as a people have a higher incidence of this disease. If glaucoma in vegetarian Jains is related to some plant toxin, the most obvious link is the milk they consume in abundance. This trend of thought fostered a series of experiments in my laboratory at Bombay during 1954-56, in which I fed goats with argemone leaves, took their milk and fed it to rhesus monkeys and succeeded in producing prolonged raised eye tension which was maintained at a raised level for many weeks. During these experiments it was found that the tension in the monkeys could be more rapidly raised and more adequately maintained when the goat's milk containing the transmitted poison was separated, the lactalbumin portion being rejected, whilst the casein-fat portion was incorporated into a low-cystein, low-vitamin A synthetic cake, which was fed to the monkeys.

The argemone herb contains seven other alkaloids besides sanguinarine and in spite of its thorns and bitter taste, is know to poison cattle and horses in Australia and the

U.S.A., and goats and donkeys in Africa, where it has been declared a "noxious weed". Arrgemone *mexicana L.* var : ochroleuca Lindl., is a favourite food of the ostrich and is browsed upon in times of drought by goats and sheep in Africa. Another thorny species, *Papaver* aculeatum Thunb., also contains sanguinarine and is considered as a good fodder in Australia. Poisoning to poultry and live-stock by argemone seeds has been recorded in Africa, the Mauritius Islands and Australia.

Protective Action of Cysteine

The goats fed on argemone leaves showed a small rise of eye tension. It is known that goats fed on D. D. T. transmit this poison in their milk without adverse effects to themselves, or to their young, although the poisoned milk is lethal when fed to rats. The removal of lactalbumin from the milk of goats fed on argemone leaves was based on my Oxford experiments which had shown that the toxic effects of sanguinarine were neutralised by nor-adrenaline, adrenaline, cysteine or B. A. L. The lactalbumin fraction in milk, which contains the bulk of the cysteine, would partially protect against the toxic actions of sanguinarine, and hence it was deliberately diminished from the poisonous milk in order to bring out its toxic effects on monkeys. These experiments also suggest the importance of giving cysteine to patients during epidemics of argemone oil poisoning which still continue to occur in India. Further, they may also explain why milk, which could transmit the toxic alkaloids from the plants, is itself portective against the toxicity, as it contains the cysteine factor. The disastrous ocular effects probably occur only when the proportion of the toxin is overwhelming, or when the poisoning is very prolonged, or the natural cysteine, nor-adrenaline or adrenaline is depleted in the patient by protein deficiency or disease.

A Carcinogenic Metabolite of Sanguinarine also Transmitted Through Milk

The detection techniques developed later in London showed that the milk of goats fed on argemone leaves contained sanguinarine and other alkaloids. This transmission experiment was repeated in London on a group of lactating rabbits that were injected with sanguinarine and subsequently milked. Sanguinarine was detected from several, even 1 ml, samples of such milk. The rabbit milk also showed a green fluorescing metabolite of sanguinarine which was found to be identical with synthetically prepared benz(c)acridine, the metabolic conversion taking place in the liver (Hakim et al., 1961 b). As benz(c)acridine has the basic molecular structure of a series of the most virulent of experimental carcinogenic hydrocarbons, according to Prof. Lacassagne from the Radium Institute at Paris, I applied both sanguinarine and benz(c)acridine on the skin of a group of mice, using his method. I also implanted paraffin pellets containing these substances into the bladder of rats, and found after several months that both substances produced, papillomas and carcinomas in mice and rats. The goat and rabbit experiments clearly proved that lactating animals if fed on sanguinarine-containing plants, could transmit glaucoma-inducing sanguinarine and cancer-inducing benz(c)acridine in their milk.

Glaucoma and the Hypothalamic Centres

Glaucoma is usually considered to be a blinding disease localised in the eye ball in which the normal tension of the intra-ocular fluid is raised by over-secretion or by anatomical, physiological or pathological obstruction of its exist channels. A great deal of research has been extended to determine the nature and location of the presumed pathological obstruction, but nothing very definite has as yet evolved out of these studies. On the other hand, there is good evidence that irreversible pathological obstruction only occurs after the disease has progressed very considerably and that the obstruction itself may be the result of a deranged physiology of the eye.

Before we can understand the pathology of glaucoma, we must investigate normal ocular physiology and specially study the problem of intra-ocular tension both in health and disease. Some of the following observations and experiments indicate that both normal and pathological eye tension are most probably under hypothalamic control from the brain. The intro-ocular tension in the majority of normal human eyes when measured by very precise instruments is between 14 and 17 mm of Hg. and shows slight increase after menstruation and delivery. If the tension is measured both in the morning and in the evening, it is found to show a small - but definite oscillation ranging from 2 to 3 mm Hg., beinq usually higher in the morning. This diurnal variation is independent of activity, sleep, food or light and corresponds to other physiological diurnal rythms such as temperature, sleep, diuresis, etc., which are regulated from the brain (Duke-Elder, 1952). When tension in one eye is mechanically raised there is a tendency for it to rise in the other. When fluid is injected into or removed from a dead eye, the pressure changes mechanically and in proportion to the volume introduced, but in the living eye, some mechanism actively resists any such change and maintains a normal or nearly normal tension within a considerable range. This also suggests a regulating nervous mechanism (Duke-Elder, 1957). It is now known that the aqueous humour is partly diffused from the blood and that some of its constituents are actively and selectively secreted and that the sympathetic nerve regulates the total blood-flow through the ciliary body and the secretion of protein, vitamin C and glucose but not of urea (Davson, 1956).

In that modern classic on glaucoma-Sir Stewart Duke-Elder's Bowman Lecture, the author emphasises that the earliest and most significant sign detectable in simple glaucoma, is not an increase in the normal tension but its instability. The diurnal range of oscillation may increase to 30 or 40 mm Hg. and yet its maximum may well be below the accepted normal level. Not only in the early stages, but sometimes during its entire course there may be no rise in tension but only an increase in its range and blindness may result long before raised tension is detected.

At this stage you will realise that the old text-book idea of pathological block in the canal of Schlemm or in the drainage channels, is quite incapable of explaining these normal physiological observations on eye tension, or those when the eye tension changes from the physiological to the pathological pattern. How could any anatomical mechanism cause more blockage in the morning than in the evening or after the menstrual period? When glaucoma has become established, how can a pathological block suddenly disappear during some part of the day so as to produce the widely swinging oscillations in eye tension? When tension has been successfully lowered by a

bypass operation, why do smaller oscillations in eye tension persist ? Then further, how is it that no anatomical block has been demonstrated in early 'simple' glaucoma, either in the canal of Schlemm or any part of the outflow canals in spite of a measurable increase in the resistance to the outflow of aqueous? In. the other type of 'narrow-angle' glaucoma, where the iris angle is narrow, there is no such resistance to outflow and the tension remains normal. Then suddenly, usually under emotional stress, the iris bulges forward and blocks up the angle alter which the tension shoots up, and an acute, catastrophic and painful attack of glaucoma is precipitated. Francois (1948) emphasises that the point of capital importance is that the closure of the angle precedes the elevation of tension, and that the tension appears only for the period whilst tine angle is closed. Francois also suggests that the hypertension is secondary, and the disease is probably situated at the diencephalon (Glaucoma, 1957). The anatomical narrowness of the angle, which may be a predisposing cause, cannot be the basic or the precipitating cause of the disease, as it is unknown in the young and. only found in adult eyes affected with glaucoma. The precipitating cause of both types of glaucoma is triggered by hormonal, emotional or other disturbances and operates from outside the eye.

It is known that electrical stimulation of the cervical sympathetic nerve lowers the eye tension and the chemical blockage of either its ciliary or stellate ganglion or systemic sympathetiocolytic drugs diminish the diurnal variations of eye tension in the normal, glaucomatous and successfully operated eyes. It is also known that visual disabilities, field defects and cupping of the discs are not necessarily produced by or related to the degree of eye tension, for some eyes can withstand very high tension for prolonged periods without any of the above changes and others rapidly and progressively succumb to such changes in spite of continuous normal tension.

These anomalies in the orthodox mechanical obstructive theory of glaucoma and observations on the influence of the autonomic nervous system on eye tension, paved the way for eminent ophthalmologists like Lagrange, Hamburger, Thiel, Dieter, Duke-Elder, Hartmann, Francois, Radovici, and others to suspect an, extra-ocular neurogenic basis for glaucoma, which Magitot localised to the diencephelon. The glaucomatous eye is not an isolated sick organ in a healthy individual but, as correctly described by Lagrange, a sick eye in a sick body.

- Experiments on the Hypothalamic Centres with Sanguinarine top

Direct experimental evidence demonstrating that eye tension is regulated from the hypothalamus came from the experiments of von Salmann and Lowenstein in America [Figure - 3] and Gloster and Greaves in England. They placed electrodes into specific areas of the hypothalamus of anaesthetized cats and found that when these areas were stimulated by an electric current, there was a rise or fall of eye tension independent of the systematic blood pressure.

Since our glaucoma patients (luckily) do not have an electrode fixed in their thalamus and since sanguinarine was already known to experimentally raise eye tension, I considered the possibility of the eye tension raising action of this alkaloid as being mediated by chemical stimulation at these hypothalamic centres. An opportunity to investigate this possibility came by working in Dr. Feldberg's department at the

National Institute for Medical Research and under his quidance. Feldberq and Sherwood (1953) have devised an ingenious technique by which a metal cannula is permanently screwed into the skull of cats [Figure - 4]. Repeated injection of drugs can subsequently be made directly into one of the lateral ventricles of the brain even in conscious animals. The very first injection of sanguinarine, in 1957, produced an acute rise of eye tension and was reported to the Physiological Society at its Oxford Meeting.

This interesting observation led to a series of experiments, first in London and later in Bombay. The very small dose of sanguinarine chloride (0.05-0.2 mg) required to raise eye tension when injected into the brain, suggested that it acted upon some exceedingly sensitive mechanism close to the cerebral ventricle and not by direct action after systemic absorption. Intravenous sanguinarine, also raises eye tension, but with fifty times the above dose and this action could also be mediated from the small amounts that reach the brain centres. This was shown by destroying the thalamus by various methods and then injecting sanguinarine by vein. When the thalamus had been destroyed, there was no rise of eye tension.

A series of 200 cats have been cannulated. A few days after recovery, their eye tension was measured with a Schiotz tonometer after instilling a few drops of 0.5% amethocaine in normal saline. My colleague and co-worker, the late Miss Freny Doctor, has measured and recorded over 30,000 eye tension. readings in conscious cats. In some animals these were taken three times daily for weeks and months. In control animals she found slightly higher reading of eye tension in the morning, showing a similarity in the diurnal variation in cats and man. For these experiments, sterile injections were made up to a volume of 0.2 ml in normal saline, at normal pH and injected at 35° C. through the rubber bung on the outer end of the cannula directly into one lateral ventricle. Injections of control substances like normal saline, glucose, urea, calcium chloride, Merlis's solution, etc., produced an insignificant or no change in eye tension. Sanguinarine chloride and other active alkaloids were injected in doses of 0.05 to 0.2 mg, the eye tension being recorded before the injection, and at intervals of 15 minutes for one or two hours after the injection. Some of the animals showed marked excitement and wild movements during the first few minutes after the injection. During the next 15 to 60 minutes no symptoms were observed in the cats, this being probably the latent period during which the alkaloid mixed with the cerebrospinal fluid in the lateral ventricle, reached the third ventricle and then seeped into the brain tissue. In most of the experiments with sanguinarine there was an increase in the eye tension, averaging between 5 to 15 mm Hg., with a maximum record of 51 mm Hg., the peak being between 60 and 90 minutes after the injection and the return to basic level after 1 to 2 hours. During the period of raised tension many of the cats showed symptoms suggesting thalamic reactions like crouching, lowering the head, licking, mewing, salivating, retching, defecating, erecting the spinal hairs and changes in temperature. The cats did not tolerate larger doses of sanguinarine and many animals died within 24 to 48 hours after such doses. Although these readings based on the Schidtz tonometer were not considered as absolute values because of the variations in the size and rigidity of the animal eye, they were important in showing the change in the tension when repeatedly measured on the same eye. Several experiments were also performed on cats under central and rabbits under local anaesthesia with a modified Davson-Purvis (1950) mirror-image manometer and the Schihtz readings confirmed and calibrated.

When injections of sanguinarine were repeated 2 or 3 times weekly; using a very small

dose, because of their toxicity, some of the cats showed a rise in the basic level of tension which could be sustained for a few weeks only. By that time the general condition of the animals deteriorated rapidly, weight fell due to refusal of food and water and either the injections had to be stopped or the cat died suddenly. It was interesting to observe [Figure - 5] that if sanguinarine had already elevated the basic tension, further injections could produce only a small additional rise. This suggested the operation of some intrinsic mechanism preventing an unlimited rise of eye tension, and added further confirmation to its hypothalamic control. Similar experiments were performed on rabbits and monkeys. Sanguinarine produced these acute rises of eye tension even after the animals were injected with decamethonium to rule out elevation of tension by contraction of the Mueller's muscle.

From an experimental point of view, it is unfortunate that when sanguinarine was repeatedly injected into the brain for a few weeks, the cats became lethargic, disinclined to eat or drink, and died suddenly. Shevalev, working in Russia on the effects of repeated systemic injections of argemone oil in cats, came across similar difficulties. I have tried single sterile injections of argemone oil into the lateral ventricle and found, as in my Oxford experiments, that the oil was very much more toxic than could be accounted for by its sanguinarine contents. Argemone seed-oil 0.01 ml, even when diluted ten times with pure arachis oil, often killed the cats within hours or days. Sanguinarine, either as base or chloride, was incorporated into paraffin tablets and operatively introduced into one lateral ventricle to act as a minute steady source of sanguinarine, but this technique proved inert. At present the alkaloid is incorporated into cholesterol tablets and placed in the brain of Loris monkeys. This technique seems promising. Permanent elevation of eye tension has been achieved over several weeks in rhesus monkeys fed on argemone or tumaria seed-oil, or by injections of sanguinarine, or best by feeding a synthetic cake into which was incorporated the casein-fat portion of the milk of goats fed on argemone leaves.

Biochemical Mechanism at the Hypothalamus

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After injecting sanguinarine or codeine into the brain of cats they sometimes showed great excitement. Reserpine (serpasil) was therefore injected systemically four hours before the brain injection. This rauwolfia alkaloid produced marked quietening in the cats and a fall of blood pressure. The subsequent injection of sanguinarine or codeine into the brain produced far greater rise of eye tension than when reserpine had not been given. This indicated that, as with electrical stimulation, chemicals injected inside the brain could produce raised eye tension even when the blood pressure was lowered. Further, Vogt had shown that reserpine markedly reduces the nor-adrenaline which is normally concentrated in the thalamus and para-ventricular areas of the cat's brain. The area of the distribution of nor-adrenaline is very similar to Sallmann's eyetension regulating areas. Nor-adrenaline probably acts as a neurochemical buffer in the thalamus and partly antagonises the effects of sanguinarine. When it is diminished by reserpine, the subsequent injection of sanguinarine or codeine is free to act and produce its maximum effect.

Several other active local hormones like acetylcholine, mono amine oxidase, and 5-hydroxytryptamine are also present and probably produced in the hypothalamus. Drugs

like sanguinarine produce their effects by preventing the formation, action or destruction of some of these local hormones.

Sympathetic Nerve Fibres Link Hypothalamic Centres & Eye Ball

When sanguinarine is injected into the brain, there is an increase of tension in the eye ball, Obviously there must be some nervous pathway linking the central mechanism and the end organ. The possibility of raising eye tension by stimulation of the various cranial nerves has been investigated in detail by Sir Stewart's research team in London, and the sympathetic seems to be the most likely connecting link. By the application of the cannulation technique, I was able to confirm directly since 1957 that the sympathetic links the tension-controlling brain centres and the eye. In a series of cats, the ventricle was cannulated and the superior cervical nerve or ganglion operatively removed *on* the right side. Horner's syndrome was produced. After recovery, sanguinarine was injected as usual through the cannula into the ventricle. On the right side, where the sympathetic had been removed, there was no rise of eye tension, whilst the tension rose as usual on the intact left side. The detailed discussions regarding the mixed nature of the sympathetic trunk and its relation to eye tension will be published later. Sanguinarine placed as a pool surrounding the superior cervical ganglion, did not block the transmission of tensionlowering effects of electrical stimulation of the pre-ganglionic sympathetic trunk.

Direct Action of Sanguinarine on the Retina

From the beginning of the experimental era, von Graefe himself observed the excavation of the optic disc without raised tension. This view, which subordinated tension, the clinical mainstay of glaucoma, has had the support of Schnabel and Duke-Elder. This suggests that the retinal changes may result from some degenerative or direct toxic action, independently of the tension. Tissue-culture experiments I carried out in the laboratory of Sir Edward and Lady Mellanby in 1953 suggested that sanguinarine had a toxic action on the retina apart from and independently of its tension-raising action. Rat embryos were aseptically removed and their eyes dissected out. One eye from each embryo was grown in a normal tissue-culture medium and the other in the medium containing various concentrations of sanguinarine or argemone oil. In another experiment the isolated retinae from embryonic rat eyes were cultured similarly in order to observe the effects of the drug on the retina isolated from other structures. Sanguinarine was also injected into the yolk of fertile eggs and the eyes of the chicks examined after hatching. These tissues were fixed and examined microscopically, and the results suggested that sanguinarine or argemone oil had some direct retinal toxicity. As this was only a pilot experiment, it would repay repetition on a larger scale.

The Age Factor in Sanguinarine Toxicity

A very valuable clue was obtained from another set of experiments regarding the

influence of age upon the retinal toxicity of sanguinarine. A group of 30 young rats were fed either on a control diet or one containing sanguinarine or argemone oil for 225 days. The microscopic examination of their eyes showed no retinal toxic effects. Lady Mellanby suggested the repetition of this prolonged experiment using old rats which she supplied from the Nutrition Laboratory. These old animals were treated like the previous group for 250 days. The subsequent microscopic examination of their eyes showed remarkable toxic changes ranging from peripheral, central, segmental to complete degeneration of the retina and along the optic nerve. Since these toxic effects were found only in the poisoned animals and only when these were old, it seemed that some biochemical mechanism was preventing toxicity in the young rats. Natural protective substances could be the sex hormones, plasma proteins, or locally produced tissue hormones like nor- adrenaline, adrenaline, acetylcholine, irin or kinin and may explain the age incidence for retinal changes in human glaucoma. Further, there may be some co-relation between the degeneraton along the optic nerve known to occur in human glaucoma as far up as the chiasma, and the degeneration of the optic nerve I was able_, to produce in old rats fed with argemone oil or sanguinarine for long periods. Tension within the eye ball cannot possibly produce changes along the optic nerve, which are usually considered as ischaemic in origin. They could possibly be toxic.

- Poppy-seeds & Poppies in the Aetiology of Glaucoma top

My co-workers found sanguinarine in a poppy-seed cake purchased in London. Fortunately the alkaloid although present in some poppy seeds is not found in all. Both poppy and argemone seeds are known to adhere to cereals tc the extent of 10 to 16%. Poppy seeds are fed to poultry as mixed grain and experimental transmission of sanguinarine to the whites of eggs has been demonstrated.

European Jews have a very high incidence of glaucoma and, as a people, consume large amounts of poppy seed and its oil. In Palestine, the seeds and oil are consumed by both Jews and Arabs, and the incidence of blindness from glaucoma is 26.3% among the Jews and 21 among the Arabs; but the incidence of blindness from all causes is eight times more among the Arabs (Sorsby, 1950). Among these two communities, therefore, although culture, hygiene or treatment diminishes total blindness, these factors have no effect on the incidence of glaucoma.

The incidence of glaucoma in European countries, where statistics are available, is definitely high in Eastern Europe and highest in Turkistan. Poppy-fumaria species are also far more numerous and prolific in these areas and their populations consume larger amounts of poppy seeds.

The highest recorded incidence of glaucoma is for the Faroe Islands in the North Atlantic. Here the predominant flora is *Papaver* naudicale L. and various species of Corydalis. Moreover, these plants, rich in sanguinarine, cover vast ice-bound and mountainous areas all over the globe and are often the predominant available fodder for milk and flesh producing animals. *Papaver* naudicale L, is known to be grazed upon by sheep in Australia where it is recognised to be toxic.

These clinical observations lend considerable support to a toxic basis for glaucoma,

particularly related to the poppy-fumaria alkaloids.

Opium Addicts & Glaucoma

The eye tension of a group of opium habitues in Formosa was recorded by Konda in 1937, who found it to be normal or slightly raised. There was a wide range in the tension of different patients but, more significantly, it showed irregular and marked variations when measured in the same patient from day to day. The latter is exactly what Duke-Elder has described as the earliest detectable change in glaucoma. I have also studied eye tension in a group of opium addicts and found a very wide range in its diurnal oscillation, and these findings will be published later on. It is significant that, in both groups of subjects absorbing some of the 25 isoquinoline alkaloids present in opium, instrumentally detectable glaucoma had set in. I would appreciate your co-operation in sending me figures of diurnal variation in eye tension in your patients who may be habitues of opium, morphine, codeine or papaverine.

The Liberation of Histamine by Poppy Alkaloids in the Aetiology of Glaucoma

For over thirty-four years DukeElder (1928, 1940) has suspected the action of a histamine-like endogen ous toxin in glaucoma which Friedenwald (1930) claimed to have found in the aqueous of patients with acute glaucoma. Kirwan (1936) and Chopra (1937) also detected a histamine-like substance in the aqueous of patients suffering from epidemic dropsy glaucoma, but its identity is questionable in the light of modern test methods (Davson, 1956). But what is very significant is that several poppy-fumaria alkaloids like morphine, codeine, papaverine, the baine and apomorphine, when injected into animals, release histamine (Feldberg et al., 1951), and that this amine experimentally changes the albumine-globulin ratio in the aqueous. Pathological changes in the tissues and eyes found in human epidemic dropsy glaucoma and those in a few experimental animals, resemble ' some of the toxic effects of histamine. When I injected 0.1-0.2 mg histamine into the brain of cats and rabbits, a rapid but small rise in their eye tension was found.

von Euler (1956) found histamine in the sympathetic nerves of the ox and a definite correlationship between this amine and nor-adrenaline in various nerves. Histamine may be the intermediary in the action of poppy alkaloids and noradrenaline in the hypothalamus.

Again, oedema is a sign of argemone oil poisoning but it can also be produced experimentally by apocodeine (Dixon, 1904). These opium alkaloids, probably by releasing tissue histamine, produce central or local effects which may have an important bearing in the aetiology of both glaucoma and oedema. This clinical and experimental comparison supports the toxic theory of glaucoma and widens its possible sources.

A New Concept of Glaucoma

Let me try and present a new concept of glaucoma. It is but a hypothesis, but as you keep it in mind. you will observe how the various experiments I have done or quoted fit into the picture. Glaucoma is a disease probably affecting nerve centres in the brain which regulate eye tension, and often leading to blindness only after many years, during which it is usually insidious and symptomless. The eye ball is the peripheral endorgan and shows secondary damage years after its nervous tension regulating mechanism is diseased. The diffused groups of nerve cells in the hypothalamus which are intimately associated with other thalamic centres such as those regulating temperature, blood pressure, etc., are constantly influenced by the conscious brain and respond to emotions of various kinds.

The association of glaucoma with auditory nerve disease and ocular hypotension with Parkinsonism More Details (Brand, 1957) suggests central nervous disturbances affecting the tension, or auditory, or tremor controlling centres. The cause of the affection of the nerve centres in the brain could be ingested toxins like sanguinarine or a host of similar alkaloids. If these are ingested insidiously and even occasionally through milk, meat, eggs, poppy seeds or drugs, the process would be intermittent and prolonged over years as is found in non-tropical glaucoma. If the poisoning is massive as when argemone oil contamintates cooking oils, an acute and relatively rapid effect is seen as in tropical epidemic glaucoma.

Coming to the eye ball, we are faced with the problem of the mechanism of normal and raised tension. The volume of aqueous could be regulated by nervous control of the secretory apparatus and the discovery of special nerve endings in the ciliary body, choroid and trabeculi lend support to the concept of a delicately balanced system which regulates blood flow into the eye somewhat like the receptors in the carotid sinus. The nervous arc may be centrally relayed from the hypothalamus or have lower relay pathways, probably in the ganglia of the sympathetic trunk. von Graefe, Becker and others have accepted the possibility of increased inflow of aqueous into the restricted capacity of the eye-ball. However, most authors attribute the raised tension to diminished aqueous outflow and Duke-Elder in his Bowman lecture illustrates its vaso-motor mechanism with great lucidity. The eye tension could also be regulated by the ionic or osmotic effects of some of the substances which are known to be actively secreted under experimental sympathetic stimulation. Acetazolamide (diamox) reduces eye tension probably by altering the osmotic relationship between plasma and aqueous and not by inhibiting carbonic anhydrase, as is usually believed (Langham, 1957).

My pharmacological experience under Professor Burn and the reminder of the classical experiments of Sir Henry Dale, Loewi and Cannon, makes me anticipate a neuro-hormonal mechanism within the eye which liberates active hormones under both local and hypothalamic nervous control, and I am conducting experiments on these lines. These hormones probably produce the known vascular effects. The very fact that von Euler (1954) found considerable amounts of nor-adrenaline and some adrenaline in the ciliary body, choroid, iris and aqueous of animals and none in the lens, vitreous or retina, suggests that these hormones are concerned with the secretory activity of the eye. These hormones are liberated on sympathetic stimulation. Irin is known to be present in the iris and an acetylcholine-like substance was found by Luco and others to be liberated into the aqueous following parasympathetic stimulation. The active secretion

of ascorbic acid into the aqueous and its concentration far above that in the plasma would prevent the too rapid destruction of noradrenaline and adrenaline and act as an oxygen carrier to the lens and cornea.

From the point of view of neurosecretion, the eye-ball can be viewed as a combined secretory and sensory organ showing embryological and phylogenetic similarities to the vestigial pineal eye, which retains its secretory but has lost its sensory light receptive function except in the rare New Zealand lizard, Sphenodon punctata, and a few other species.

The mechanism of permanently elevated eye tension and the pathological changes in the eye has been suggested by Duke-Elder (1957) and has analogies with oscillating systemic blood pressure which preceeds permanent hypertension. We have much to learn in this unknown field.

Possibility of Endogenous Toxins in the Aetiology of glaucoma

Another very important but hitherto unexplored possibility is the toxic effect on the brain centres by endogenous poisons formed in the body of the patient by inborn or induced errors of metabolism, and valuable clues in its search may be found from the biosynthesis or metabolism of sanguinarine. Sir Robert Robinson and Manske suggest that poppy-fumaria plants build up the amino-acid tyrosine, change it by steps to nor-laud anosine and eventually to numerous alkaloids including sanguinarine. The animal body used the same amino-acid to synthetize thyroxine and melanin and the two animal alkaloids nor-adrenaline and adrenaline. Adrenaline and sanguinarine, built up from the same precursor by animal and plant, are active biological antagonists in man, sanguinarine producing many of its effects by substrate competition with natural local hormones. In the animal body, sanguinarine is metabolised to the carcinogenic benz(c)acridine, both substances are closely related to phenanthrine and phenanthridine and to a number of active substances like cholesterol, vitamin D, sex hormones and experimental carcinogens. It is possible that some natural or induced error in amino-acid metabolism produce a glaucoma-genetic toxin.

The Legacy of Glaucoma

We have come a long way from the days of the Hippocratic school of physicians who saw eyes "green like the sea" and called them 'glaucous'. The torch of knowledge was handed down the ages from Greece to Arabia and Iran. But unfortunately this epoch was dominated by a hypothetical philosophy of pathology consisting of the 'elements' and 'humours', and the term 'aqueous humour' is a modern reminiscent of this period. After this came the Western renaissance of instrumental ophthalmology, in which the theory of the causation of glaucoma swung away from that of a generalised derangement of the body 'humours' to the localised obstruction of the 'aqueous humour'.

In the pre-instrumental centuries, the symptoms of eye pain and diminished vision and the signs of hardness and opacity of the eye were discovered. All of us now know that these signs and symptoms are usually the terminal events in the course of this insidious

disease. With the help of modern instruments we can now measure the degree and changes of hardness, examine the depths of the anterior chamber, see and chart the progressive destruction of the retina, medically control or surgically relieve the increased pressure. But most of us forget that both raised tension and retinal changes are absent for many years during the developing stages of glaucoma; or so insidious that, in eight hundred thousand persons in the United States of America alone and there must be millions in the whole world-glaucoma has already set in without either patient or doctor being even aware of the disease. Most of us break the very first rule for the early detection of glaucoma by taking a solitary 'measurement of the patient's eye tension, and that too at an evening clinic-just the time when the tension is minimum or absent ! When for the convenience of diagnosis, treatment or prognosis, we divide primary glaucoma into the insidious simple "open angle" type and the catastrophic "narrow" angle type, we are observing two totally different phenomena in the eye, but behind both may be a common derangement of the nervous tension-regulating mechanism which operates remotely from the brain and is affected by emotion or disease.

The dynamics within the eye ball cannot chart the prognosis of your patients, for even when you operate and succeed in relieving one symptom which is the tension, their vision often continues to degenerate. Even in advanced countries like the United Kingdom, where early detection and adequate treatment are available, nearly 60% of cases proceed to blindness. So once again we must return, with the humility that comes from knowledge, to the theory of the derangement of the generalised functioning of the nervous system for a better understanding of the problem of glaucoma. We must not forget that, in spite of the enormous collection of observations and experiments in hospitals and research laboratories throughout the world, we know next to nothing about the cause of glaucoma, very little about the mechanism of raised tension, and can only alleviate and not radically cure the disease.

The story of glaucoma has not ended. Many chapters have yet to be written. A few years ago nothing was known about the cause of glaucoma. Now at least we know about argemone 'oil glaucoma and experimental glaucoma with sanguinarine. We have also discovered that sanguinarine poisoning is very much more widespread than we had ever thought possible and that it can occur from drugs, milk, eggs and meat. Other plant alkaloids similar to sanguinarine can also be responsible for toxic glaucoma. On the other hand, we have found natural protective substances that can prevent their toxicity. In the experimental investigation of sanguinarine, we have found that it can produce raised eye tension for prolonged periods when injected or fed by certain techniques and that extremely small doses can produce acute rise of eye tension by affecting the nerve centres in the hypothalamus. We have not yet succeeded in producing a permanently elevated level of eye tension by this means.

The eradication of glaucoma-the second largest cause of blindness in the world-requires your active and intelligent help and co-operation. An open mind, the patience and alertness to inquire into and investigate the presence of toxicity in your patients, the keenness to detect the disease in its early stages, the skill in treating them and above all, the sympathetic understanding of the patient's nervous background which is at the root of the disease, will all be required. Some amongst you could contribute even more by experimental research. But whatever be your contribution, let it brighten some cloud in the story of glaucoma and let it give hope and eventual assurance of the joy of seeing.[54]

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